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The Joint Action of DDT and Ethyl Alcohol upon Anopheline Larvae in Bioassay Suspensions[1]

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Several factors affecting the evaluation of insecticides against anopheline larvae were enumerated by Krusé *et al.* (1952). These modifying effects included numbers of larvae per test, stability of the test suspension, and the volume of test suspension. In a later report (Hawkins & Kearns 1956) the stability of DDT suspensions, and consequently larval kill, was shown to be dependent upon the ionic environment in the suspension. Cardboard cartons being a good source of ions are much less suitable as test containers than glass dishes which have a more stable surface. Recently it has been found that the amount of the DDT solvent, ethanol, used to produce the suspension also exerts an effect on larval kill. The results of this study are presented here.

MATERIALS AND METHODS.—Three methods were used to prepare DDT suspensions varying in ethyl alcohol content. Percentages of solvent were calculated on a volume per volume basis. For those suspensions containing 1% DDT solvent, 1 ml. of each dilution of a series of DDT solutions was added to 99 ml. of distilled water. Suspensions containing 0.1% DDT solvent were prepared in a slightly different manner. First, 1% alcoholic suspensions were prepared by adding 1 ml. of DDT solution to 99 ml. of water, each containing 10 times the amount of DDT required in the test suspension. Since these suspensions also contained 10 times the amount of alcohol needed in the test suspension, a 10-ml. aliquot of each of these suspensions was pipetted into 90 ml. of water to make the final test suspensions having an alcohol concentration of 0.1%. The suspensions containing 0.01% alcohol were prepared in a similar manner except that each initial 1% alcohol suspension contained 100 times the amount of DDT and alcohol needed in the test suspension; thus, a 1-ml. aliquot was added to 99 ml. of water to make the final test dilution.

Those tests in which the alcohol content decreased parallel to the dosage were prepared from initial suspensions containing either 5 μg. or 500 μg. DDT and 1% solvent in 100 ml. of distilled water. From the suspension containing 5 μg. DDT, aliquots containing the required amount of DDT were taken and made up to 100 ml. with distilled water ("5 μg."). In a similar manner test suspensions were prepared from the suspension containing 500 μg. DDT. However, to obtain this lower solvent concentration, 1 ml. of the 500 μg. DDT suspension was first diluted to 100 ml. with distilled water, and the final test suspensions were prepared from this auxiliary dilution by transferring aliquots to distilled water ("500 μg."). To find the percentage of alcohol in each 100 ml. test suspension of the "5 μg." series multiply the p.p.m. value by 20; while for the "500 μg." series multiply the p.p.m. value by 0.2.

To condition the larvae of *Anopheles quadrimaculatus* Say with ethyl alcohol, they were held for 4 hours in a 2% solution and were then transferred to distilled water for 15 minutes so as to at least partially cleanse them of the alcohol solution. The larvae were then introduced into the DDT suspensions and mortality counts were made after 24 hours.

Early fourth stage larvae of *A. quadrimaculatus* were used as the test insects. They were reared in the insectary at a temperature of 75±2° F. on a diet of powdered dog chow. The criterion of death for the treated larvae was their failure to move upon being touched. Control mortality was corrected for by means of Abbott's formula. The curves were fitted by sight to the plotted points. Because of the qualitative nature of the results, no statistical analysis was made of the data. The test dishes were 90-mm. "pyrex" glass crystallizing dishes. For the introduction of both solutions and suspensions, the material was pipetted beneath the surface while the water was being vigorously stirred. Tests were conducted at a temperature of 75±2° F.

RESULTS.—In colloidal suspensions of DDT, the toxicity of the DDT appears to be related to the amount of the initial DDT solvent (ethyl alcohol) present. Though no data are presented here, it has been found that similar results are obtained when the solvent is either acetone or isopropyl alcohol. The results of parallel bioassays with DDT in which the ethyl alcohol concentration at all dosages was 1.0, 0.1, and 0.01% are illustrated in figure 1. The curves show that in some way the solvent enhances the toxicity of the DDT. Because of the shape of the curves, the LD_{90} dose is equal in suspensions containing 1.0 and 0.1% alcohol (0.01 p.p.m. DDT), but the LD_{90} dose in the presence of 0.01% alcohol is twice this value. However, at the 50% kill level, the dose doubles with each decrease in alcohol concentration.

The results of dosage response tests with the "5 μg."

PAID PAPER.

[1] Accepted for publication April 17, 1956.

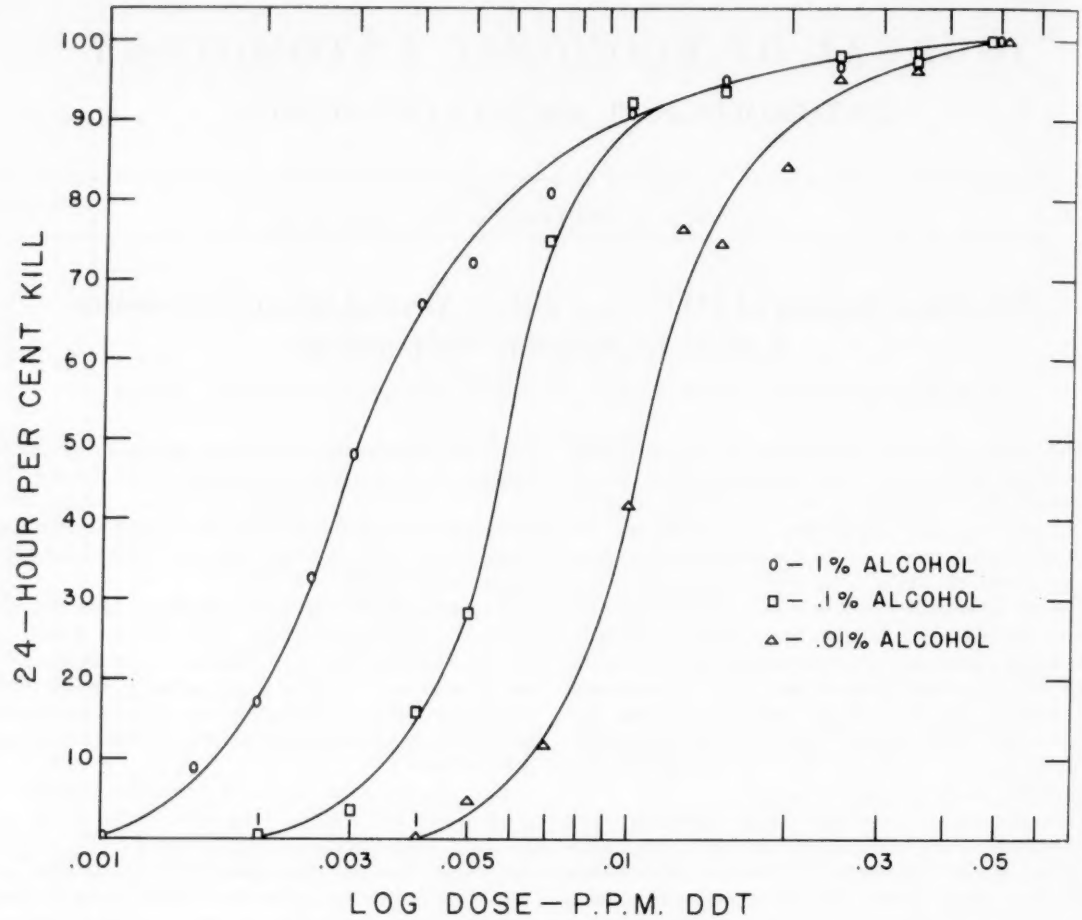

FIG. 1.—The dosage response of *Anopheles quadrimaculatus* larvae in DDT suspensions when the concentration of the initial DDT solvent (ethyl alcohol) varies between series of tests.

and "500 μg." DDT formulations are shown in figure 2. In these tests the decrease in DDT dosage was paralleled by a proportional decrease in alcohol content. The exposure of larvae of *A. quadrimaculatus* to 2% ethyl alcohol solutions for 4 hours, followed by exposure to test series of the "5 μg." and "500 μg." suspensions, increased the larval kill (figure 2). At the highest concentration of DDT and alcohol, the conditioned and unconditioned larvae show about equal tolerances to DDT, but, with each lowering of the DDT-alcohol concentration, the conditioned larvae show an increased susceptibility to DDT. Also, the effect of conditioning is much more outstanding in the series of suspensions containing less alcohol.

DISCUSSION AND CONCLUSIONS.—From the data presented here, it is concluded that the effectiveness of DDT against anopheline larvae in colloidal suspensions of DDT is correlated with the concentration of the initial DDT solvent, *i.e.*, ethyl alcohol. This conclusion is supported by the evidence that three roughly parallel mortality curves were plotted when the alcohol concentration was 1.0, 0.1, and 0.01%. Each decrease in alcohol content shifts at least a portion of the curve toward the higher dosages in a constant amount. This constancy is considered to be evidence that the alcohol is a factor controlling the effectiveness of the DDT. Further evidence of the alcohol's effect is given by the data from tests in which the alcohol concentration decreased parallel to DDT dosage. Here again lower kills resulted with each decrease in solvent concentration. However the kills are consistently lower than one would predict on the basis of the solvent content alone (figure 1). Thus other factors must be affecting the action of the DDT in the "5 μg." and "500 μg." formulations but are as yet unknown. These pieces of evidence are still further supported by the increased susceptibility to DDT exhibited by larvae conditioned in an alcohol solution prior to exposure to the DDT. The alcohol may either "combine" with the cuticle in some way so the DDT can penetrate more rapidly, or it may affect some internal physiological system so that DDT is more toxic. The usual practice of subjecting control larvae to a solution equal in solvent content to that of the test suspensions cannot be considered a critical test of the solvent's action. Rather, the joint action of the solvent and toxicant must be determined if bioassay results of moderate significance are to be obtained. Either in testing for resistance or in the screening of

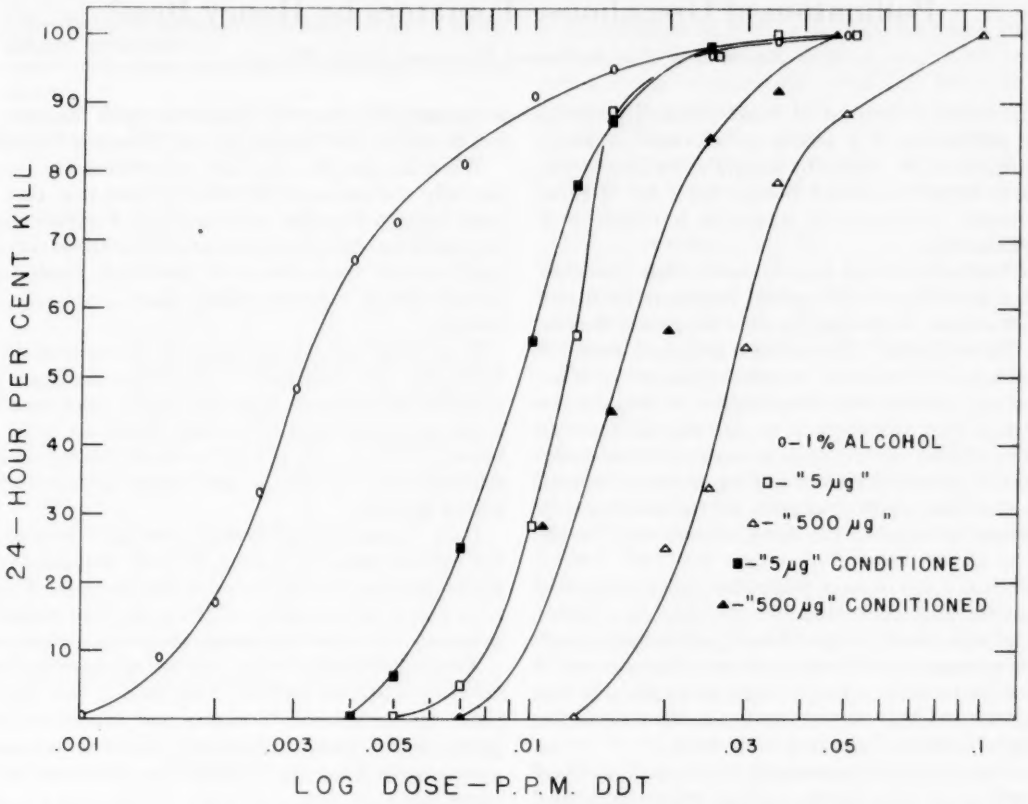

FIG. 2.—The dosage response of *Anopheles quadrimaculatus* larvae in DDT suspensions when the concentration of the initial DDT solvent (ethyl alcohol) decreases parallel to dosage, with and without pre-conditioning of the larvae in a 2% alcohol solution, compared to the response in the presence of a constant alcohol concentration.

compounds for insecticidal activity variations in bioassay formulations could change larval kill sufficiently to lead to erroneous conclusions.

SUMMARY.—The toxicity of DDT dispersed in colloidal suspensions by the replacement of solvent method is correlated with the concentration of the initial DDT solvent in the suspension. The LD_{50} of *Anopheles quadrimaculatus* larvae in DDT suspensions containing 1.0, 0.1, and 0.01% ethyl alcohol increases by a factor of two with each ten-fold decrease in alcohol concentration. However there is no parallel increase in the LD_{90} when the percentage of alcohol decreases from 1.0 to 0.1; but the LD_{90} is doubled when the alcohol is further lowered to 0.01%. Test suspensions were prepared by dilution of aliquots of an initial suspension so that the alcohol concentration decreased parallel to the DDT concentration. As before, larval kill decreased with each lowering of the alcohol concentration, but to a greater degree, indicating the influence of other, but unknown factors. The toxic action of DDT is increased by conditioning the larvae in 2% alcohol solution prior to placing the larvae in DDT suspensions containing small amounts of alcohol. The results emphasize the necessity for studying this joint action of solvent and toxicant if with mosquito larvae more quantitative bioassay results are to be obtained.

REFERENCES CITED

Hawkins, W. B., and C. W. Kearns. 1956. The stability of a DDT suspension. Bull. Ent. Res. (in press).

Krusé, C. W., G. F. Ludvik, and W. B. Hawkins. 1952. Factors affecting evaluation of insecticides against *Anopheles* larvae. Jour. Econ. Ent. **45**(4): 598–601.

Pollination of Greenhouse Tomatoes by Honey Bees[1]

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Tomato plants grown out of doors commonly obtain adequate pollination. The plants are vibrated by winds and the blossoms are visited by insects of various kinds, particularly solitary bees and bumble bees. All of these natural factors contribute to successful fertilization of the fruit blossoms.

It was learned 50 years ago, however, that tomatoes grown in a greenhouse need special treatment to insure proper pollination. According to Ohio Extension Service Bulletin SB-10 (1954), "Greenhouse tomatoes must be hand pollinated in order to insure a good set of fruit. This involves tapping each flower cluster to transfer the pollen grains from the anthers to the stigma. Since all flowers in a cluster do not open at once, it is necessary to continue tapping the cluster as long as there are open blossoms. . . . The electric vibrator is the most widely accepted tool for tapping the flower cluster early in the season."

Inasmuch as 9,000 or more plants are grown per acre in a greenhouse and approximately 10 blossom clusters develop on each plant, the problem of pollinating an acre of greenhouse tomatoes involves a tremendous amount of labor. For that reason a project was undertaken at the Ohio Agricultural Experiment Station to investigate the possibility of inducing bees to do this work.

Bees are being used commercially for the pollination of a wide variety of crop plants, but the writer has found no published record of their use in pollinating tomatoes. Occasional reports have been observed similar to that of H. W. Schneck (1928), a horticulturist at Cornell University, who stated: "Honey bees which facilitate pollination of greenhouse cucumbers, do not work well on tomato blossoms, probably because of the peculiar structure of the flower and the absence of nectar."

A factor that must be borne in mind when attempting to pollinate tomatoes is the fact that the amount of pollen which reaches the stigma of a tomato blossom has a marked effect on the size and shape of the resulting fruit. When only a few pollen grains reach the stigma, small fruits and often irregularly shaped fruits result. These are usually very solid and have few if any seeds.

Sunshine, relatively high temperatures, and a dry atmosphere are important factors in the formation, liberation, and germination of pollen. Pollination is difficult in periods of cool and cloudy weather. However, the stigma of a tomato blossom is thought to be in condition to receive pollen for a period of 4 or more days.

Experiments in Honey Bee Pollination.—In the entomology greenhouse at the Ohio Agricultural Experiment Station bees have been used experimentally as pollinators of tomatoes for six consecutive crops. The bees are maintained in the greenhouse throughout the year, except for relatively short periods when a crop is being removed or the plants are being treated with insecticides.

Inasmuch as tomato blossoms contain little or no nectar, the bees in the greenhouse are given constant access to a sugar solution made by mixing sugar and warm water in equal parts. The bees are also fed a pollen supplement when tomato blossoms are not available, but this is discontinued when the tomatoes are in bloom in an effort to induce the bees to visit the blossoms for pollen.

When the project was first undertaken the bees were partially starved with the idea in mind that they might work tomato blossoms more actively if sufficiently hungry and if no other food were available. Observations and yield records show, however, that best results are obtained with a vigorous colony that is actively rearing brood.

In early fall and late spring when the vents of the greenhouse are open and flowers of various kinds are readily available out of doors, bees will usually work such blooms more vigorously than the tomato blossoms in the greenhouse. However, the period in which pollination is most difficult and bees can be most useful occurs during the winter months.

Both Caucasian and Italian bees have been used, but the former apparently work at lower temperatures and hence are more satisfactory for this purpose. They have been observed a number of times working tomato blossoms actively when the temperature was as low as 65° F.

Two small rooms in the entomology greenhouse separated by a narrow hallway were used in this study. One room accommodates 72 plants and the other 84 when grown in the manner commonly followed in commercial greenhouses. The variety WR Globe has been used in all cases.

The plants are arranged in rows with six plants in each row. Half of the rows in each room are marked with a red streamer and the blossom clusters on the rows so marked are vibrated with an electric vibrator at intervals of 1 or 2 days. No effort is made to pollinate the remaining plants, although some accidental pollination occurs when the plants are being pruned and trained up the supporting twine, and when fruits are picked.

Records have been taken of the percentage of the blossoms that set fruit, and the number of fruits produced in each cluster, but because of the relatively large number of small fruits that develop when flowers are improperly pollinated, the most reliable record obtained has been the weight of the tomatoes produced on each plant. In taking the latter record only fruits that weigh 2 ounces or more are included.

For the fall crop of 1952 and the spring crop of 1953, bees were maintained in both rooms. Yield records showed an increase in yield of 1 pound per plant in one case and 2 pounds per plant in the other from bee and hand pollination over that effected by bees alone. However, the bees had access to all plants and their influence on the yield from the plants on which the clusters were vibrated mechanically, could not be determined.

For the four succeeding crops bees were maintained in only one of the two rooms. While the first three of these crops were being pollinated the doors between the two rooms were open constantly, and bees were often observed in both rooms although they worked the tomato blossoms in the room where the hive was located much

[1] Accepted for publication December 13, 1955.

Table 1.—Yields of tomatoes obtained by different methods of induced pollination.

METHOD OF INDUCED POLLINATION	FRUITS PER PLANT	WEIGHT PER FRUIT (OZ)	POUNDS PER PLANT
	Fall Crop—1953		
None[a]	14.0	2.8	2.5
Bees only	16.0	3.1	3.0
Vibrator[a]	24.6	3.3	5.1
Vibrator+bees	26.9	3.4	5.8
	Spring Crop—1954		
None[a]	47.4	2.7	8.0
Bees only	41.9	3.8	9.9
Vibrator[a]	37.3	5.0	11.7
Vibrator+bees	39.0	4.9	12.0
	Fall Crop—1954		
None[a]	23.0	3.5	5.0
Bees only	31.9	3.5	7.0
Vibrator[a]	32.3	3.8	7.6
Vibrator+bees	38.0	3.6	8.5
	Spring Crop—1955		
None	28.5	3.1	5.5
Bees only	27.1	5.6	9.5
Vibrator	26.1	5.1	8.4
Vibrator+bees	29.4	6.0	11.1

[a] Although no beehive was located in the room where these plants grew, a small number of bees from the hive in the adjacent room worked a portion of the blossoms.

more effectively. During the period in which the spring crop of 1955 was being pollinated the doors between the two rooms were closed in order to confine the bees to one room. This provided a more adequate check on the work of the bees.

The yield data obtained are summarized in table 1. The two fall crops were small primarily because the plants were removed relatively early and therefore produced fewer blossom clusters than the plants grown in the spring. However, due to better growing conditions and longer days, the tomatoes produced in the spring were also somewhat larger than those grown in the fall.

In all cases the highest yield was obtained where the bees worked the blossoms that were also pollinated with the electric vibrator. This increased yield, apparently due to a more complete pollination, averaged 1.1 pounds per plant.

The work of the bees was more effective in the spring crop of 1955 than in any other crop. This is apparently due to the vigor of the colony and to the fact that the bees were confined to the one room and therefore pollinated no blossoms in the other room. In that crop pollination by bees alone resulted in a higher yield than pollination with the electric vibrator.

The number of fruits produced per plant reflects the degree of pollination in the fall crops, but not in the spring crops. A plant with relatively few fruits in the early clusters tends to produce more blooms in the later clusters. Because the spring picking seasons extended over longer periods, blooms that were unintentionally pollinated while picking the early fruits, produced small fruits later.

It is interesting to note that size of fruit is closely correlated with size of crop. Since it is known that poor pollination results in small fruits with few seeds, this is rather good evidence that the differences in crop size are due primarily to differences in pollination efficiency.

BEES IN A COMMERCIAL GREENHOUSE.—A vigorous colony of honey bees was also used in the plant house of a commercial greenhouse near Elyria, Ohio, while the spring tomato crop of 1955 was in bloom. This planthouse covered approximately $\frac{1}{9}$ acre of ground. One row of tomatoes comprising 63 plants was pollinated only by bees. The remaining plants in the planthouse as well as those in the main range, which comprised $2\frac{1}{2}$ acres, were pollinated in the usual way.

Although yield records were not obtained, counts of the number of fruits per cluster, and measured diameters of more than 1,000 fruits in the first four clusters indicated that the plants pollinated only by bees produced approximately the same size crop as the plants in the main range. Fruits that were pollinated with the electric vibrator and also by bees averaged slightly larger and were more numerous than those in the main range. The grower was well pleased with the results and plans to use bees in the main range when the next spring crop is in bloom.

CONCLUSION.—Bees can be induced to work the blossoms of greenhouse tomatoes during the winter months when no other blossoms are available. The data indicate also that bees may increase the degree of pollination sufficiently on plants pollinated by hand to warrant their use commercially. However, colonies used for this purpose must receive sufficient attention to maintain them in a healthy and vigorous condition.

Further work is needed under commercial conditions before statements can be made concerning (1) the number of colonies needed per acre, and (2) the possibility of bees replacing all or part of the labor now needed to pollinate greenhouse tomatoes.

REFERENCES CITED

Anon. 1954. Growing greenhouse tomatoes. Ohio Ext. Service Bull. **SB-10.**

Schneck, H. W. 1928. Pollination of greenhouse tomatoes. Cornell Agr. Expt. Sta. Bull. **470.**

Two Mymarid Egg Parasites Attacking *Typhlocyba* Species in California[1]

MIR S. MULLA,[2] *University of California, Berkeley*

During 1953–1954, as work on the biology of *Typhlocyba prunicola* Edwards and *T. quercus* (Fabr.) progressed (Mulla & Madsen 1955), two hymenopterous egg parasites, *Anagrus epos* Gir., and *A. armatus nigriceps* Gir.,[3] were reared out of the eggs of these two leafhoppers. These two egg parasites play a relatively important role in regulating the natural populations of *T. prunicola* (prune leafhopper) and are less important on *T. quercus* (oak leafhopper). Parasitized eggs have light yellow to red orange color, and can be easily seen through the leaf epidermis.

ANAGRUS ARMATUS NIGRICEPS GIRAULT.—This variety was first described by Girault in 1915, reared from the eggs of *Typhlocyba rosae* (L.). Since its first description, this variety has not been mentioned in the literature. As both the rose leafhopper and prune leafhopper, its known hosts, have a wide distribution, it is possible that the parasite might also have a wide distribution. It is also possible that it may have other hosts as well. Its effectiveness as an egg parasite is not well known. During the course of the biological studies of the two leafhoppers only 12 females and one male of this variety were obtained as compared to 167 male and female adults of *A. epos*. *A. armatus nigriceps* was only reared from the summer eggs of *T. prunicola* and was not found among the parasites that emerged from overwintering eggs of *T. prunicola* and *T. quercus*. This limited information thus gives no indication of how the parasite overwinters.

The male and female (Fig. 1) adults are robust bodied with crimson-red compound eyes. The ovipositor is stouter than that of *A. epos* and the antennal club of the female has more prominent hairs and hair tubercles than does that of the latter species. The larval development and manner of emergence is apparently like that of *A. epos*.

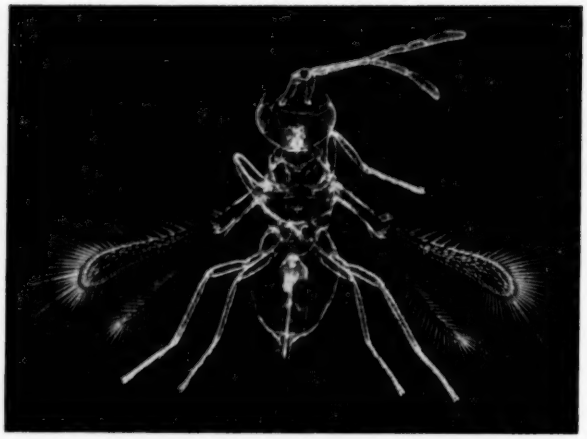

FIG. 1.—Adult female of *Anagrus armatus nigriceps* Gir. Ventral view, ×72.

FIG. 2.—Adult male of *Anagrus epos* Gir. ×72.

ANAGRUS EPOS GIRAULT.—This species was first described by Girault in 1911. He did not record the host, since the adults were captured on a window pane in Illinois. His description applies very well to the specimens obtained in California. He gave the length of the females as 0.35 mm. and that of the males a little less. After its first description, this parasite has been mentioned in the literature on several occasions. It has been reported to parasitize the eggs of *T. rosae*, *T. pomaria* McAtee, and *Erythroneura pleua* Beamer in eastern part of the United States (Ackerman 1919, 1931, McConnell 1931). According to Mackie (1931), *A. epos* appeared for the first time in considerable numbers in some California vineyards, parasitizing the eggs of the grape leafhopper, *Erythroneura elegantula* Osb. In 1932, Lockwood observed 25 to 95% eggs of *E. elegantula* parasitized in some California vineyards. He stated that this parasite is probably the cause of reducing the host broods from the normal three to two.

The adult male and female of *A. epos* are 0.30 to 0.35 mm. in length, and are difficult to see with the unaided eye. They are quite active and the females locate host eggs with the club of their antennae. The ovipositor is serrate at the posterior end and extends slightly beyond the abdomen when in the sheath. The compound eyes are usually dark, but light red in newly emerged individuals. Ocelli are three in number and of a ruby color. The males (Fig. 2) have 13 antennal segments, while the females have only 9.

[1] Accepted for publication December 8, 1955.

[2] Acknowledgement is due Dr. Harold F. Madsen for his valuable criticism and suggestions during the progress of this work, and to Dr. R. L. Doutt, who read the manuscript and made suggestions for its improvement.

[3] Determined by Dr. B. D. Burks, Insect Identification and Parasite Introduction Section, Entomology Research Branch, U. S. Dept. of Agriculture, and the first species also by Dr. R. L. Doutt, Division of Biological Control, Univ. of California, Albany.

Life History.—The complete life history of *A. epos* as well as most of the other members of the genus is not well known. Observations made on the life history and life stages of *A. epos* during the course of this work were brief and therefore would not present a complete picture. However, these may prove useful in further studies of the parasites belonging to this group.

In California *A. epos* overwinters as a half-grown larva (histeriobdelid stage). The histeriobdelid stage (Fig. 3) is represented by several larval forms. The cephalic lobes are quite long and retractile in the early larval stages. It is not known, as to what function the posterior sucker-like structures perform. These can also be invaginated or evaginated to a lesser degree. The heavily sclerotized prongs which are thought to be mandibles are invariably present and are easily observed (Fig. 3).

The larvae pupate inside the host eggs and become flattened (Fig. 4). The pupae are of a light yellow color first and darken to brown color as the emergence time of the adult approaches. At this stage they occupy the whole lumen of the host egg. The unemerged adults lie flat on their backs with their mandibles directed upwards against the bark or epidermis (Fig. 5). They make a round hole in the bark or leaf tissue through which they emerge. The emergence holes (Figs. 4 and 6) remain visible for 2 to 3 weeks after emergence, before they become plugged up.

Emergence has been observed throughout all of the growing season of the trees (i.e. from late March to the early part of November). *A. epos* possibly produce more than one generation in a single brood of the host eggs.

PARASITISM IN THE FIELD.—During 1953–54, the parasitism of the eggs of *T. prunicola* and *T. quercus* varied from orchard to orchard (Table 1). As a whole the egg parasitism of *T. quercus* was lower than that of *T. prunicola*. During the months of August and September only a few overwintering eggs of *T. prunicola* are deposited in the twigs, and those of the summer generation in the leaves are almost all hatched out or already parasitized. It appears to be at this time that *A. epos* attacks the eggs of *T. quercus* to some extent.

In 1953 the summer generation eggs of *T. prunicola* were heavily parasitized and the nymphal and adult populations considerably reduced. The emerged adults of *A. armatus nigriceps* were fewer than those of *A. epos*, and on this basis it is assumed that the portion of eggs parasitizied by the former species is smaller, though this

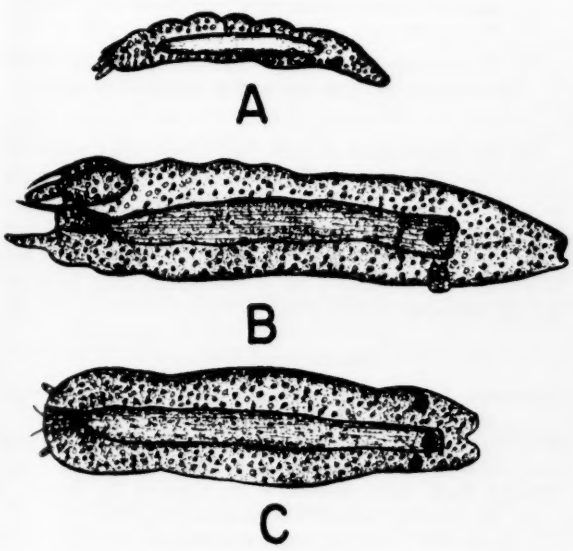

FIG. 3.—Various histeriobdelid larval stages of *Anagrus epos* in the eggs of *T. prunicola*. A, earlier, B, later, and C, before pupation. ×70.

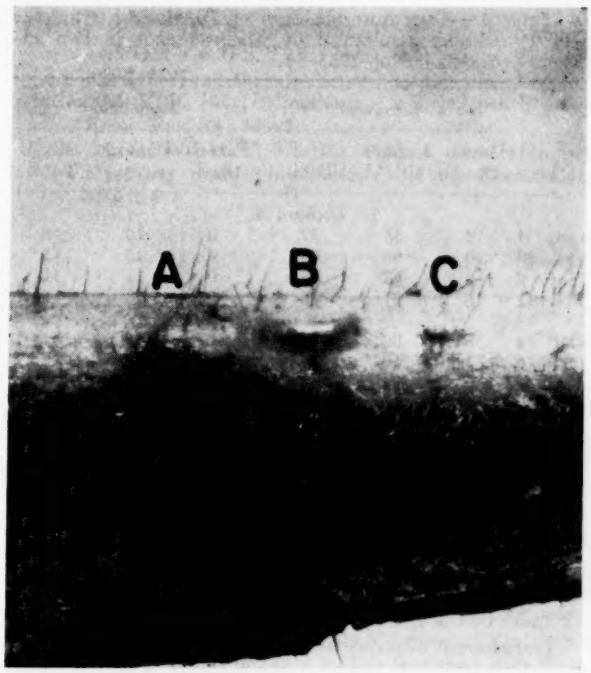

FIG. 4.—Emergence hole (A), unparasitized host egg (B), and pupa (C) of the parasite *A. epos*. ×15.

FIG. 5.—Fully developed adult of *A. epos* near emergence time. The dark color of the eyes is apparent. ×15.

Table 1.—Overwintering eggs of *Typhlocyba prunicola* and *T. quercus* parasitized by *Anagrus epos* in two adjacent orchards during 1953–54.

Date	*T. prunicola*			*T. quercus*		
	Parasitized	Unparasitized	Total	Parasitized	Unparasitized	Total
			Orchard A			
Oct. 4	2	55	57	0	15	15
Oct. 20	0	66	66	0	15	15
Nov. 2	3	45	48	0	11	11
Mar. 11	15	51	66	0	14	14
			Orchard B			
Oct. 4	8	32	40	16	166	182
Oct. 20	4	40	44	8	88	96
Nov. 2	12	32	44	2	55	57
Mar. 11	20	32	52	4	71	75

may not be true in all cases. A rapid increase in percentage parasitism between June 20 and July 20 (Fig. 7) is indicated when the host egg population density also rapidly increased (Fig. 8). Thereafter, the parasitism increased gradually until in early part of September, 95% or more of the few remaining eggs were parasitized.

The amount of parasitism follows the trend of host egg population (Fig. 8). At first, the increase in parasitism is slower than the increase of host egg population density. But after 2 to 3 weeks the parasites cope with the rapid increase of host egg population density. After the peak of host egg population is attained, the level of parasitism keeps nearly steady, and there are few eggs that are not parasitized. At this time the parasites start working on the overwintering eggs of *T. quercus* and later on the overwintering eggs of *T. prunicola*.

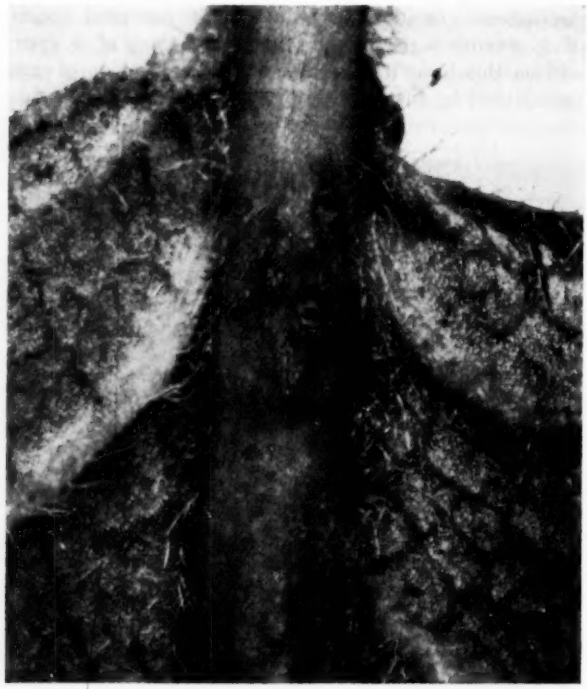

Fig. 6.—Emergence holes of the egg parasites of *T. prunicola* in the midrib of the prune leaf. ×15.

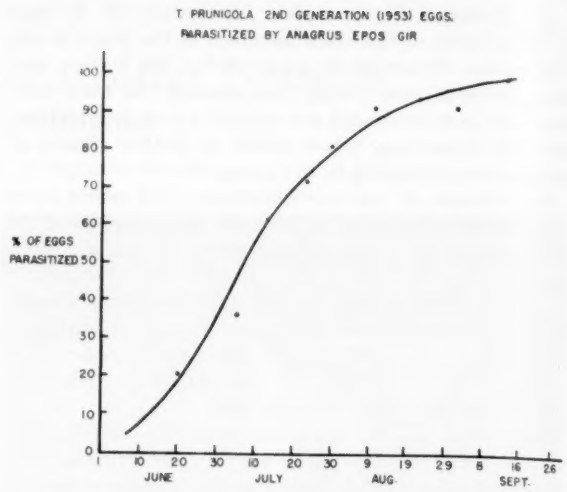

Fig. 7.—Egg parasitism of *T. prunicola* (summer generation) by *A. epos* and *A. armatus nigriceps* during 1953 season. In September parasitism is quite high but the actual number of parasitized eggs present was low.

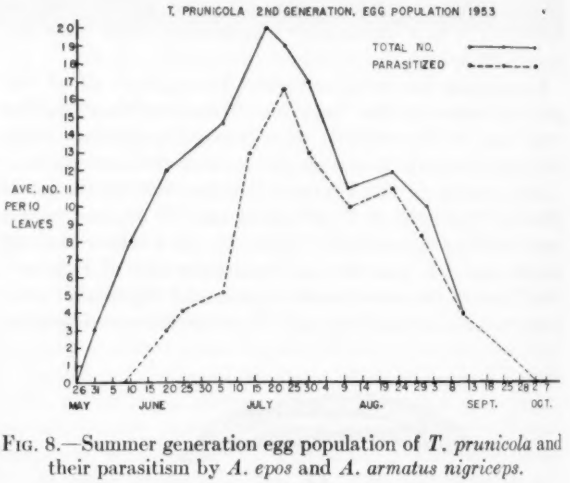
T. PRUNICOLA 2ND GENERATION, EGG POPULATION 1953
TOTAL NO.
PARASITIZED
AVE. NO. PER 10 LEAVES
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
26 31 5 10 15 20 25 30 5 10 15 20 25 30 4 9 14 19 24 29 3 8 13 18 23 28 2 7
MAY JUNE JULY AUG. SEPT. OCT.

Fig. 8.—Summer generation egg population of *T. prunicola* and their parasitism by *A. epos* and *A. armatus nigriceps*.

The relationship of *A. epos* to its hosts may not be as simple as stated above. It is possible that other hosts such as San Jose scale (reported by Annand 1942 and Cox 1942, as a host from Virginia), the rose leafhopper and others may enter into the picture. Further work will be needed to elucidate some of the factors that influence the effectiveness of this parasite.

References Cited

Ackerman, A. J. 1919. Two leafhoppers injurious to apple nursery stock. U. S. Dept. Agr. Bull. **805.**

Ackerman, A. J. 1931. The leafhoppers attacking apple in the Ozarks. U. S. Dept. Agr. Tech. Bull. **263.**

Annand, P. N. 1942. Report of the Chief of the Bureau of Entomology and Plant Quarantine (**1941–42**): 1–60.

Cox, J. A. 1942. Effect of dormant sprays on parasites of the San Jose scale and Terrapin scale. Jour. Econ. Ent. **35:** 698–701.

Girault, A. A. 1911. Description of North American Mymaridae with synonymic and other notes on described

genera and species. Amer. Ent. Soc. Trans. 37: 253–324.

Girault, A. A. 1915. Some new Chalcidoid Hymenoptera from North and South America. Ann. Ent. Soc. Amer. 8: 272–8.

Lockwood, S. 1932. The grape leafhopper in California with special reference to its control. Calif. Dept. Agr. Monthly Bull. 21: 375–93.

Mackie, D. B. 1931. Report of the Division of Entomology and Pest Control. Calif. Dept. Agr. Monthly. Bull. 20: 745–61.

McConnell, H. S. 1931. A leafhopper injuring peach. Jour. Econ. Ent. 24: 560–1.

Mulla, M. S., and H. F. Madsen. 1955. A new leafhopper attacking prunes in California. Jour. Econ. Ent. 48(4): 476.

Observations on the Alfalfa Weevil in Delaware, 1955[1]

H. E. MILLIRON[2]

The information herein, on the alfalfa weevil, *Hypera postica* (Gyll.), supplements that reported for 1953 and 1954 by Milliron & MacCreary (1955).

WINTER SURVIVAL.—Litter samples, of approximately 1 peck each, were taken from an alfalfa field at the University of Delaware Farm, Newark, on March 9 and 15, 1955, and from a similar field near Bombay Hook on March 10 and 16. A Berlese funnel was used to extract the numbers of weevils recorded in table 1. Three possible reasons for the paucity of adults were: (1) low winter survival, (2) unsuitable technique for measuring spring adult population, and (3) little or no surface activity at the time of sampling. The latter seems least plausible, since there was ample evidence of spring egg-laying activity beginning March 16. Low winter survival was indicated by the results of the following limited experiment. On October 22, 1954, a few pupae within cocoons and a few adults were placed on the surface of soil covered by typical litter in a circular metal container, 3½ inches high and with screened top and bottom, which was buried to such a depth that the soil level inside corresponded with that outside this cage. When examined on March 7, 1955, all individuals were recovered as dead adults, one apparently infected with the fungus, *Beauveria globulifera* (Speg.). However, a large number of specimens placed in a variety of microhabitats should be observed before reliable conclusions can be drawn regarding the effect of Delaware winters on hibernating weevils.

EGG STUDY.—Dead, dried alfalfa stems were obtained at random from fields in early March at the locations mentioned above. A sample consisted of a minimum of 100 stems, each of which was scrutinized with a stereoscopic microscope. When an oviposition-puncture was detected, the egg cavity was located by dissection, its contents removed, studied and recorded. Examination of a total of 415 stems collected at Newark was made on March 9, 15, 22 and 30 and only 10 egg cavities were found. Therefore, the meager data obtained at that location are not included in table 2. The fact that eggs were more difficult to find at Newark than at Bombay Hook was probably due to the presence of fewer adults during the preceding fall, or to lower temperatures that curbed egg laying in early spring at the former location.

From the data presented in table 2, a range of from one egg puncture in 20 stems on March 10 to one in six on March 30 was derived, or an average of one in every 13 stems. The increase resulted partly from a progressive improvement in sampling, which was reflected in more cavities and greater quantity of eggs in stems chosen on the last two sampling dates.

The average number of eggs (hatched and unhatched) per cavity was 10.5 (tables 2 and 3). Since no fresh eggs were obtained prior to mid-March, all dark ones dissected until approximately that time were considered as having passed the winter. Subsequently, special importance was attached to the dark color of eggs and to the aged appearance of the cavity in assigning material to the overwintered category. Some dark eggs in cavities examined on March 30, after hatching was well along in the field, were classified with doubt, unless they were associated with old egg shells, and/or the general appearance of the cavity indicated it had been made sometime the preceding fall.

On March 10, three of the five cavities located (67%) contained overwintered eggs; while of those found on March 16, 22% had old eggs and 33% had recently-laid eggs (table 2). Twenty-eight cavities were dissected on March 23, showing 61% with old eggs and 25% with ones that were new. The 18 cavities found on March 30 revealed 70% with overwintered eggs and 17% with those freshly-laid. Expressed in another way, the percentages of old and new eggs found on these several dates were as

Table 1.—Alfalfa weevils from litter samples. Delaware, 1955.

SAMPLE	DATE	NUMBER OF ADULTS
University Farm, Newark	March 9	1
University Farm, Newark	March 15	3
Bombay Hook	March 10	0
Bombay Hook	March 16	2

PAID PAPER.

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[2] Assistant Research Professor, Entomology.

Table 2.—Alfalfa weevil eggs in dead alfalfa stems. Bombay, Hook, Delaware, 1955.

Date	Stems	Number of Cavities					
		Total	With Eggs	With Egg Shells	With Egg Shells and Unhatched Eggs	With Old[a] Eggs	With New Eggs
March 10	100	5	3	2	0	3	0
16	150	9	5	2	1	2	3
23	400	28	23	2	2	17	6
30	100	18	16	2	1	13	3
Totals	750	60	47	8	4	35	12

[a] Overwintered eggs.

follows: March 10, 100 and 0; March 16, 50 and 50; March 23, 76 and 24; March 30, 82 and 18 (table 2). The data pertaining to the last two collections indicate a reverse of the expected rate of egg production under normal conditions. This is possibly explained by the retardation brought about by fluctuating, unusually-low temperatures during the last 2 weeks of the month.

This same weather factor also apparently affected the rate of hatch, which is expressed in percentage eclosion observed (table 3). The rate of hatch in 1953 and 1954 proceeded at a progressively rapid pace, once it began in March; at best, the 1955 figures show a gradual increase between the second and third weeks of March and very little throughout the remainder of the month. At Dover, the nearest U. S. Weather Bureau Station (about 7 miles distant), the average maximum temperature for the first half of March, 1955, was 59° F. (table 5), and the average minimum 37° F., with 3 days at 32° F. or below. The average maximum during the second half of the month was 56° F., and the average minimum 35° F., with 6 days at 32° F. or below. Higher temperatures in early March favored embryonic development, which was abnormally checked by colder weather after the middle of the month. Likewise, the adverse temperatures had a marked effect on the viability of eggs, particularly those that overwintered and began to embryonate during the first half of March, after which viability dropped from 82 to 48%.

It was difficult to determine the viability of some of the dark, turgid, overwintered eggs by superficial examination at the time they were dissected from stems. In order to ascertain the degree of possible error in evaluating such material, 91 additional eggs were obtained from surplus stems in the sample collected at Bombay Hook on March 10. For convenient handling, these were divided into two lots of 37 and 54 and held in vials at room temperatures for hatching, which was completed by March 15. Fifty-six per cent of the first lot hatched and 44% of the second, making a total average of 50%. If these had been considered viable eggs, the error for the first lot would have been 44% and for the second 56%, or an average error of 50% in this instance.

Another lot of 27 overwintered eggs was studied with respect to time of hatch in relation to density of color, when removed from the cavities on March 11. Seven of these were definitely dark, but without signs of advanced embryonic development, while the remaining 20 were drab yellow. Five of the dark eggs hatched on March 15, and the remaining two on the following day. Eleven of the yellowish eggs hatched on March 16, and the remaining nine on March 17. The darker eggs hatched in advance of the lighter ones by 1½ to 2 days.

Hatching under 1955 field conditions at Bombay Hook was not detected in dead stems until March 16. Samples of growing alfalfa tips from the same location were brought to the laboratory at frequent but irregular intervals, beginning on March 10, and examined microscopically for larvae. The first newly-hatched larva was located on March 23. Subsequent to that date, they became very common.

LARVAL POPULATION.—At irregular intervals from early April through August, larvae at different locations in selected fields 2 or more years old were collected in a 15-inch sweeping net and recorded. Sometimes, inclement weather, agronomic practices, or spraying operations made selection of substitute fields imperative. When a selected field had been sprayed, counts were discontinued there and made elsewhere, until after the effect of the treatment in the original field was no longer evident, or until after the treated growth had been harvested. Also, results of field examinations were not recorded during the latter half of May, because of the lack of time necessary to count carefully very large larval populations obtained at all locations in even a single sweep. Sufficient data were collected, however, to demonstrate the general population trend throughout most of the season, exclusive of that period (table 4).

The weevil was considerably more abundant than in 1954, and the larval peak occurred at, or very shortly after, the middle of May. This was comparable to the situation in 1953, but unlike the early peak recorded in April, 1954. Average maximum and minimum temperatures during the 1953–55 critical spring period at Dover (Kent County) are given in table 5. Most of the fields under observation were located in Kent and northern Sussex Counties.

Unusually cool weather during the second half of March is believed to have been responsible, in part, for the delayed 1955 population peak. April conditions in 1954 were more favorable than those of that month in either 1953 or 1955, and induced the population to develop an early peak. In 1953, the rate of increase was slowed down in April—not March, as in 1955—by adverse temperatures, so that the peak did not occur until about the second week of May. Temperatures in April

Table 3.—Viability of alfalfa weevil eggs in dead alfalfa stems. Bombay Hook, Delaware, 1955.

Date	Number of Eggs				Per Cent of Eggs		
	Total	Hatched	Viable, Unhatched	Nonviable	Hatched	Viable	Nonviable
March 10	43	6	37	0	14[b]	100	0
16	69	14	45	10	20	82	18
23	311	43	129	139[a]	25	48[a]	52
30	208	31	85	92[a]	27	48[a]	52
Totals	631	94	296	241			

[a] Embryonic development began earlier at this location. High mortality believed due to cold temperatures during last week of month.

[b] Determined from old egg shells present in aged cavities; therefore, hatch presumed to have occurred before onset of winter.

Table 4.—Average alfalfa weevil population per 25 sweeps in fields 2 or more years old. Delaware, 1955.

PERIOD	LARVAE	ADULTS	SAMPLES
April			
2nd week	5	11	11
3rd & 4th weeks	353	11	8
May			
1st & 2nd weeks	1551	21	6
June	60	29	6
July	33	3	3
August			
1st & 2nd weeks	4	3	5
3rd & 4th weeks	0	2	3

appeared to be a more important factor regulating rate increase than were those in March. The population trend for the remainder of the season was similar to that in 1953.

PARASITISM.—Two hundred and sixty adult *Bathyplectes curculionis* (Thom.), an effective hymenopterous parasite of the alfalfa weevil in the Western United States were liberated 2½ miles north of Bridgeville on May 27, 1954, through the courtesy of the Entomology Research Branch, U. S. Department of Agriculture, under very favorable conditions. Attempts to recover the parasite at this point began April 25, 1955, when approximately 4,000 full-grown host larvae were brought to the laboratory and allowed to spin more than 2,000 cocoons. Careful examination of 1,258 of these did not reveal a single parasitized pupa, and the remaining cocoons were examined superficially without detection of any parasites. By May 9, cocoons were present by the thousands on all parts of alfalfa plants, chickweed, dried grasses, dead stems of weeds, and in litter on the ground at the liberation point. A thorough search, covering several square yards, did not disclose any evidence of parasitism. A collection of 134 cocoons, taken on that date and brought to the laboratory for closer examination, also was free of parasites. If *B. curculionis* is present in Delaware, it is certain to be scarce and, as yet, has had no noticeable effect on the abundance of its host.

Table 5.—Average temperatures. Dover, Delaware.[a]

	MARCH				APRIL			
	First Half		Second Half		First Half		Second Half	
YEAR	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
1953	52	33	59	41	61	44	67	45
1954	51	34	59	36	65	41	75	50
1955	59	37	56	35	68	45	66	49

[a] Computed from Climatogical Data, Maryland and Delaware, U. S. Weather Bureau, March and April, 1953–55.

CONCLUSIONS.—A small-scale attempt to carry pupae and adults through the winter in an outside container resulted in complete mortality before the approach of spring. Few adults were obtained from litter samples examined during the first half of March, 1955, possibly indicating low adult survival, inadequate technique, or little surface activity at the time samples were taken.

Eggs in dead, dried, alfalfa stems were easier to locate at Bombay Hook, Delaware, than at Newark. Egg cavities ranged from one in 20 to one in six stems at the former location, and the average number of eggs per cavity was 10.5. Spring-deposited eggs were not found until mid-March. Both spring egg production and the rate of hatch of overwintered eggs were retarded by low temperatures during the second half of March. This weather factor also greatly increased mortality of newly-hatched larvae and/or the embryonic stage, and resulted in decreased viability of eggs that had passed through all, or part, of the winter.

Hatching under field conditions was detected in stem samples as early as March 16, and the first recently-hatched larva in new alfalfa growth was found on March 23.

In 1953, larvae were most abundant during the first half of May and, in 1954, during late April. They were more abundant in 1955 than in the preceding year, and the population peak occurred at, or shortly after, mid-May. While temperature conditions in March may affect the rate of larval increase, there appeared to be a more direct correlation between April temperatures and the time that the first generation larval peak occurred.

All attempts to recover *Bathyplectes curculionis*, introduced in 1954, were negative.

REFERENCE CITED

Milliron, H. E., and Donald MacCreary. 1955. The alfalfa weevil in Delaware, 1953–54. Jour. Econ. Ent. **48**: 283–9.

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Attractiveness of Sweetclover Varieties to the Sweetclover Weevil[1]

M. C. Wilson and R. L. Davis, *Purdue University, Lafayette, Indiana* and B. A. Haws and H. L. Thomas, *University of Minnesota, St. Paul*

For a period of several years the sweetclover weevil, *Sitona cylindricollis* Fahr. has been a serious problem in the midwest to farmers trying to establish and maintain a sweetclover stand. Investigators have conducted research on the biology and control of this insect, Bird (1947), Munro *et al.* (1948, 1949), Herron (1953), Haws (1954), and Haws & Holdaway (1954). Wilson (1951), and Wilson & Barber (1954) showed that effective control could be obtained with insecticide-fertilizer combinations and that under moderate infestation good sweetclover stands were obtained without insecticides by increasing plant vigor and growth through the addition of fertilizer.

In 1953 investigators in Minnesota and Indiana initiated field studies to determine if there were any differences in the susceptibility or attractiveness of various sweetclover varieties to the weevil. Haws (1954) reported significantly less damage to Common White and Common Yellow than to most other varieties. Since similar observations had been made in Indiana, the investigators in both states collaborated so that results could be more readily compared. In 1954 and 1955, the same seed source was used in both states. Common Yellow and Common White sweetclover seed, supplied by Purdue University, was seeded in both states and seed of some other sweetclover varieties was also interchanged.

Field Studies.—*Methods.*—Techniques of seeding and evaluating sweetclover varieties for weevil damage have varied in the two States. In Indiana, the varieties were drilled with a small grain in early April at the rate of 10 pounds of seed per acre. In 1953 the sweetclover was drilled in wheat which had been seeded the previous fall. In 1954 and 1955 the legume was drilled together with oats. In all tests varieties were replicated six times in a randomized block. Plots were 10×14 feet in size.

In Minnesota the clover seed was sown alone in 1953 and 1955 but oats was included as a nurse crop in 1954. Each year all varieties were replicated three times in a randomized block design with plots 8×30 feet in 1953 and 1954 and 10×30 feet in 1955.

Beginning at the time of the emergence of the sweetclover, regular observations were made in Indiana on the weevil damage and on sweetclover stand and vigor. The study in Minnesota has been largely confined to weevil damage. Two techniques of determining the amount of damage by the weevil have been used. In Indiana a rating scale of 1 to 9 was used. Ratings of 1 to 3 indicated little damage, 4 to 6 average, and 7 to 9 indicated severe injury. In these tests 20 plants were selected at random from each plot and each plant was rated individually.

In Minnesota the amount of leaf area destroyed by the weevil was estimated on a specified number of leaves. This method of evaluation results in a quantitative measure of injury that is comparable from year to year but the procedure is slower than the rating system used in Indiana. In 1955 Minnesota and Indiana investigators worked together and used both methods to evaluate the same plots in Crookston, Minnesota. There was close agreement in the results obtained by the two observers.

Results and Discussion.—(1) *Weevil Damage.*—A comparison of weevil damage to sweetclover varieties in Minnesota and Indiana is shown in table 1. For easy comparison, all data have been proportionately converted to the 1 to 9 scale previously described. In 1953 and 1955, Minnesota and Indiana data showed that some average differences in weevil injury among the varieties were significant at the 1% level, and Indiana data in 1954 also showed average differences that were significant at the 1% level. Although field data did not show significant differences in Minnesota in 1954, the varieties, with only one exception, showed the same trend as in other studies. Common Yellow, Common White, and Spanish, though they ranked average, were greatly superior to Madrid and Evergreen, a trend in both states. Wisconsin A-46 ranked the same as it did in Minnesota in 1953 and 1955. Wisconsin S-65 was the only variety out of place in 1954, for reasons that were not discovered.

In general, as the average of 15 tests shows, the common sweetclovers were less attractive to the weevils. Seven tests show that Spanish has been superior in Minnesota and good in Indiana, while Madrid and Evergreen have been more attractive than the average to the weevils in both states. Madrid and Evergreen have appeared less favorable in Minnesota than in Indiana. In Indiana, the two new Wisconsin selections, though agronomically very good, have consistently been more attractive to the weevils than other varieties in both field and greenhouse tests. This reaction does not appear to have existed in the 1955 data designated by footnote d in table 1. The more favorable rating in 1955 in Indiana was possibly due to the stand. Wisconsin A-46 was seeded at twice the rate of the other varieties since its germination was considered low. However, germination was heavy and a stand count showed nearly twice as many plants as there were in the other variety plots. Therefore, damage may appear less since it was distributed over a greater number of plants. The two Wisconsin selections have ranked much better in Minnesota than in Indiana.

Minnesota studies have been conducted at Crookston in northwestern Minnesota, approximately 600 miles north of Lafayette, Indiana. While supporting evidence was not obtained, it is possible that growth differences of the varieties in these widely separated areas might account for some of the apparent inconsistencies in the amount of weevil injury observed. There were obvious differences in the intensity and time of weevil infestation between the two areas in 1955. Differences in the time of year the plots were evaluated might also have complicated the results. In Indiana injury was evaluated several times during the season but these data were recorded only once each year on the plots in Minnesota.

(2) *Sweetclover Stand Vigor.*—It is important that uni-

[1] Purdue University Agricultural Experiment Station Journal Paper No. 929. Accepted for publication December 12, 1955.

Table 1.—Comparison of sweetclover weevil damage to sweetclover varieties.[a]

VARIETY	NO. OF TESTS	MINNESOTA Field Studies 1953	MINNESOTA Field Studies 1954[b]	MINNESOTA Field Studies 1955	INDIANA Field Studies 1953	INDIANA Field Studies 1954	INDIANA Field Studies 1955	INDIANA Greenhouse 1954	AVERAGE
Common White (Minn.)	3	1.0	5.0	4.5	—[c]	—	—	—	3.5
Common Yellow (Minn.)	3	1.5	5.0	5.0	—	—	—	—	3.8
Common White (Ind.)	5	—	—	3.5	1.0	1.0	1.0	1.0	1.5
Common Yellow (Ind.)	4	—	—	2.5	1.0	1.5	3.0	—	2.0
							(Average of Commons)		2.5
Erector	2	2.0	—	—	—	—	3.0	—	2.5
Spanish	7	2.5	4.0	1.0	4.2	2.8	5.5	5.0	3.6
Brandon Dwarf	2	3.5	—	—	—	—	4.0	—	3.7
Evergreen	7	6.5	9.0	7.2	6.5	4.8	4.0	4.5	6.1
Wisconsin A-46	7	5.5	5.0	4.0	8.0	9.0	4.0[d]	9.0	6.6
Wisconsin S-65	6	—	1.0	5.0	9.0	6.0	9.0	8.5	6.4
Madrid	7	8.5	8.0	9.0	6.4	2.0	9.0	6.8	7.1
Nebraska	1	9.0	—	—	—	—	—	—	9.0

[a] Data proportionately converted to 1–9 scale (1–3 superior or low damage, 4–6 average, 7–9 severe).
[b] 1954 Minnesota differences non-significant, 1953 and 1955 significant at 5% level. Indiana data significant at the 1% level.
[c] Not included in test.
[d] Damage to Wisconsin A-46 not severe because of heavy stand. (Twice as many plants as other varieties).

form stands be obtained in studies of varietal attractiveness to an insect, as a variation in stand might greatly affect the results. A variety may appear to have less injury than another but the lesser injury may have resulted because the plot had more plants per unit area than another variety and therefore the injury might have been diluted.

In Indiana stand and vigor were evaluated several times during the season by using a rating scale and by counting the number of plants per unit area. Agronomists at Purdue University obtained most of the data which are shown in table 2 during the last week in May, 6 to 8 weeks after seeding. Most of the damage by sweetclover weevils that have overwintered is completed by the last of May and the surviving stand is established. The new generation of weevils that emerge in July seldom do serious damage in Indiana, but in Minnesota these weevils may destroy stands.

These data suggest several conclusions.—(a) In Indiana the common sweetclovers which had the least amount of weevil feeding produced the best stands, but Evergreen, which consistently showed average injury or more, produced a stand less than average.

(b) The Wisconsin selections, A-46 and S-65, which were consistently damaged the most by the weevil in Indiana, produced good stands with exceptionally high vigor in 1954 and 1955. In 1953 the Wisconsin selections seeded in wheat produced a good stand, but a stand inferior to Common and inferior to both Common and Evergreen in vigor. Wilson & Barber (1954), pointed out that weevil damage was worse and stands were more difficult to obtain when sweetclover was seeded with wheat as a companion crop than with oats. It appears that the great amount of weevil feeding in the plots of the Wisconsin selections in 1953 combined with the greater competition of the wheat for sunlight and nutrients had an adverse effect on vigor. In contrast, even though these varieties were severely chewed in 1954 and 1955, sturdy plants were produced when oats was used as a companion crop. The presumed ability of the Wisconsin selections to tolerate more weevil injury than some of the other varieties tested may be associated with their inherent vigor.

Table 2.—Comparison of stand and vigor of sweetclover varieties in field plots at Lafayette, Indiana.[a]

VARIETY	1953 Stand	1953 Vigor	1954 Stand	1954 Vigor	1955 Stand	1955 Vigor
Common White	1.9	3.5	3.0	3.0	2.1	5.7
Common Yellow	—	—	2.5	3.4	1.7	5.3
Erector	—	—	—	—	4.2	6.3
Brandon Dwarf	—	—	—	—	6.0	7.5
Spanish	8.0	6.8	3.4	2.4	4.8	3.5
Evergreen	6.0	4.1	5.8	3.4	6.8	5.3
Wisconsin A-46	4.0	6.3	3.8	1.2	1.5	2.0
Wisconsin S-65	4.0	6.9	1.4	1.2	3.8	3.3
Madrid	3.1	4.7	2.7	2.5	4.3	3.5
L.S.D. at 5% level	1.5	2.1	1.3	1.5	1.3	2.0
1% level	2.0	2.8	1.7	1.9	1.8	2.6

[a] 1953 companion crop, wheat; 1954 and 1955, oats.
Rated on 1 to 9 scale: 1 to 3, superior; 4 to 6, average; 7 to 9, low.

GREENHOUSE STUDIES.—During the winter of 1954–55 greenhouse studies on 10 varieties were conducted at the Purdue Agricultural Experiment Station.

Methods.—In each test the seed was pre-germinated and then planted as follows: Six flats measuring 18×24 inches were used, each as a replicate. Varieties were randomized with one row per variety in each flat. Ten vigorous seedlings were planted per row, making a total of 100 plants spaced equally in each flat. Three cages 3×3 ×3 feet were used. Two flats were placed in each cage and 200 weevils were introduced when the plants had developed three leaves. Artificial light was provided from 4 P.M. to 2 A.M. by a 200-watt light placed over each cage.

The weevils were allowed to feed for 10 days before the flats were removed. Each individual plant was then rated for weevil damage using the Indiana rating method previously described.

Results.—Table 3 shows the distribution of damage ratings in a total of 100 plants per variety in a greenhouse study. In general, damage to Common White,

Table 3.—Individual Varietal Plant Ratings of Sweetclover Weevil Damage in Greenhouse Studies 10 Days After Infestation (1954–55).[a]

Variety	Number of Ratings per 100 Plants			Weevil Selectivity	
	Light	Average	Severe	Rejected	Destroyed
Florence	4	42	54	2	40
Spanish	20	30	50	7	28
Madrid	19	33	48	17	33
Wisconsin A46	0	37	63	0	52
Wisconsin S65	14	30	56	2	39
Evergreen	5	49	46	2	25
Israel (6141)	24	35	41	20	26
Common White	28	42	30	20	12
T72A2	24	24	52	11	35
R204 B11	28	35	37	20	17
Average	17	36	48	10	30

[a] Plants rating 1 to 3, light; 4 to 6, average; 7 to 9, severe.
Rejected = number of plants rated light exhibiting very slight or no feeding by the weevil.
Destroyed = number of plants rated severely damaged by the weevil, beyond recovery.

Spanish, Evergreen, and the Wisconsin selections followed a trend comparable to Indiana field studies.

Comparison of Field and Greenhouse Studies.—Six of the varieties studied in the greenhouse were also studied in the field. Results of comparison of weevil damage in field studies at Crookston, Minnesota, and in field and greenhouse studies at Lafayette, Indiana, are shown in table 4.

In examining the data by analysis of variance each test was used as a replicate. The results showed that average differences in the percentage of plants destroyed and those with slight or no damage were significant at the 5% level. The range of damage in Minnesota field plots was much less than in the Indiana plots; Minnesota stands were unusually heavy and the percentage of plants destroyed very low in comparison with the number destroyed in Indiana. The greater loss of stand in Indiana may have been associated with the more severe test of the varieties there where the clover was grown in competition with a small grain. The decreased vigor associated with the competition may have reduced survival of the clover plants.

Table 4.—Comparison of the distribution of sweetclover weevil damage among six varieties in Minnesota field tests with Indiana field and greenhouse tests in 1955.

Variety	Per Cent of Plants Destroyed				Per Cent Plants with Slight or No Damage			
	Field Tests		Greenhouse		Field Tests		Greenhouse	
	Minnesota	Indiana	Indiana	Average	Minnesota	Indiana	Indiana	Average
Common White	3.3	11.7	12.0	9.0	28.0	27.0	20.0	25.0
Spanish	1.7	18.3	28.0	16.0	58.0	15.0	7.0	26.6
Evergreen	18.3	27.0	25.0	23.4	17.0	2.0	2.0	7.0
Wisconsin A-46	16.6	30.0	42.0	29.5	2.0	12.0	0.0	4.6
Wisconsin S-65	18.3	61.7	39.0	39.7	22.0	1.0	2.0	8.3
Madrid	53.3	40.0	33.0	42.1	0.0	3.0	17.0	6.6
L.S.D. at 5% Level				21.0				14.3

The results obtained in field and greenhouse studies in Indiana showed a close correlation ($r = .877$ for 4 degrees of freedom).

Summary and Conclusions.—Studies conducted during 1953, 1954, and 1955 in Indiana and Minnesota indicated that differences existed in the amount of weevil injury sustained by the sweetclover varieties compared. None of the varieties studied escaped injury but the Common Whites and Common Yellows consistently sustained less injury than other varieties tested. In general, Spanish was damaged less by the weevil than other varieties with the exception of the Commons. The performance of some varieties in respect to weevil injury was different in Minnesota than in Indiana. It was noted that there were more weevils in Minnesota than in Indiana and that the critical times of infestation in the two areas were different. It is suggested that differences in plant development in Minnesota and Indiana might also be related to differences in weevil injury in the two places.

New Wisconsin selections A-46 and S-65 showed superior agronomic qualities in Indiana, but were more attractive to the sweetclover weevil than most other selections.

There was a close correlation between results of field and greenhouses studies in Indiana.

References Cited

Bird, R. D. 1947. The sweetclover weevil, *Sitona cylindricollis* Fahr. Canad. Ent. **79:** 5–11.

Haws, B. A. 1954. Sweetclover insects. Proc. North Central Branch Ent. Soc. Amer. **9:** 30–32.

Haws, B. A., and F. G. Holdaway. 1954. Sweetclover weevil and its control in Minnesota. Univ. of Minn. Ext. Folder **180.**

Herron, J. C. 1953. Biology of the sweetclover weevil and notes on the biology of the clover root curculio. Ohio Jour. Sci. **53**(2): 105–12.

Munro, J. A., and R. L. Post. 1948. Parasites to aid in the control of the sweetclover weevil. Sci. **108:** 609.

Wilson, M. C. 1951. Control of the sweetclover weevil in Indiana. Jour. Econ. Ent. **44:** 792–6.

Wilson, M. C., and S. A. Barber. 1954. The influence of the sweetclover weevil and soil fertility on sweetclover stands. Jour. Econ. Ent. **47:** 117–22.

Toxicity of Malathion to Poultry and Their Ectoparasites[1]

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The search for a safe insecticide with a broad spectrum of effectiveness against the ectoparasites of poultry has been pursued by many investigators over the years. Such a material must be acaricidal as well as insecticidal, and should be applicable directly to the birds as well as to their housing. It should, moreover, be adaptable for use in the varied types of installations commonly used by poultrymen, ranging from deep litter houses to individual wire cages.

Recent reports, citing effective control of poultry lice and mites obtained with malathion, indicate a need for further investigation of this promising compound. Vincent *et al.* (1954) obtained effective control of the northern fowl mite, *Bdellonyssus sylviarum* (C. & F.), in a limited series of tests by treating the birds with 4% malathion dust. In further experiments using the dust as a deep litter treatment at the rate of 1 pound per 20 square feet and a small handful in the nest boxes, great reduction of mites resulted, but not complete control. Furman *et al.* (1955) reported effective control of the chicken mite, *Dermanyssus gallinae* (DeG.), using 1 pound of a 2% malathion dust for 20 square feet of litter coupled with a 1% malathion house spray.

Harding (1955) reported effective control of the northern fowl mite on hens obtained by dusting litter with a 4% malathion dust at the rate of ½ pound per 20 square feet of surface plus a small handful in each nest box. This treatment was ineffective in controlling the mite on roosters, but hand dusting the latter gave effective control. Moore & Schwardt (1954) in New York state obtained excellent control of three species of chicken lice with a single roost spray of 3% malathion emulsion, while a 1% house spray eliminated the common chicken mite. Vincent *et al.* (1954) found that 50 parts per million of malathion in the diet of laying hens caused no reduction in egg production or hatchability during a 10-day feeding period. No off flavors were detected in the eggs. Dicke *et al.* (1955) noted no toxicity in 1-month-old turkeys permitted to run on manure and wire treated weekly with 2% malathion emulsion.

The present report was designed to determine the safety factor for poultry treated directly with malathion emulsions, and also to determine the effectiveness of malathion for control of the fowl tick, *Argas persicus* (Oken), and the chicken body louse, *Menacanthus stramineus* (Nitz.), under California conditions. Included in this report are observations on control of the chicken mite with malathion. Data on control of the northern fowl mite, with malathion sprays and dusts applied directly to the birds are being presented elsewhere. A future report, closely related to the present one, will present the results of analyses of poultry tissues and eggs for malathion residues.

TOXICITY OF MALATHION TO POULTRY.—A commercial malathion emulsion concentrate[2] containing 5 pounds of actual malathion per gallon was used in preparing poultry dips. Control birds were dipped in xylene emulsions of varying concentrations up to a maximum of 5.5%, using *Triton X-100* as an emulsifying agent. This concentration of xylene is equivalent to the highest concentration of solvent encountered in the test dips.

Birds were dipped by submerging all but the head in the liquid for approximately 20 seconds, ruffling the feathers by hand to ensure thorough wetting. The birds tested included White Leghorn hens, approximately 9 months old, and turkey poults, 12 to 16 weeks of age. Five chickens were used per test dip; the dips consisted of 1, 4 and 10% malathion emulsions. Turkeys in groups of four birds per test dip were treated in 0.5, 1.0, 2.0 and 4.0% malathion dips; two turkeys were dipped in 10% malathion. A single mature goose was also treated in a 4% malathion dip.

Both chickens and turkey poults successfully withstood dipping in 1% malathion emulsions, although three of five treated chickens showed temporary abnormality. Mortality occurring over a range of 1 to 7 days resulted at higher concentrations, with pronounced abnormality or death occurring more quickly with increasing concentration. The LD-50 for turkeys appeared to be in the range of a 2% malathion emulsion applied as a dip. A 4% malathion emulsion dip produced 100% mortality in all birds treated, including the adult goose, which died within 24 hours following dipping. In interpreting these results it should be emphasized that birds dipped in emulsions are probably exposed to several times the quantity of toxicant than would be the case were the birds sprayed with an equal concentration of the material at the field tested rate of 1 gallon per hundred birds.

Control birds which were dipped in 5.5% xylene emulsions showed little abnormality in gross appearance or actions, although laying hens ceased egg production and showed extensive scabbing of the skin when sacrificed 10 days following treatment. The skin of birds dipped in malathion emulsions containing a comparable quantity of solvent did not show evidence of such marked inflammation. Autopsy of turkeys moribund or dead following malathion dips showed little gross pathology attributable to the treatment. Slight mottling of the liver and hyperemia of the brain were suggestive of toxic effects.

FIELD TESTS FOR CHICKEN LOUSE CONTROL.—Tests for effectiveness of malathion roost sprays in controlling poultry lice were conducted on two commercial scale poultry ranches in Alameda County, California. The fowl were White Leghorns harboring an essentially pure infestation of the chicken body louse. Ranch "A" utilized wire-floored cages with an approximate floor area of 36 square feet per unit, each containing 30 to 40 birds. The wire floors were supported by 1-inch by 3-inch wooden strips, with the wire resting on the narrow side of the strips. Ranch "B" was equipped with deep litter floors of relatively large size harboring up to several hundred birds per unit. Each unit had a roost "porch" area on one side which was elevated over raised wire floors so that droppings from the roost area fell outside the deep litter room.

[1] Accepted for publication December 12, 1955.

[2] American Cyanamid Company.

In each unit to be treated several representative birds were examined for lice, the degrees of infestation recorded, and the birds marked with leg bands. Birds with over 100 lice were considered as heavily infested, 25 to 100 lice as moderately infested, 1 to 25 lice as lightly infested. Since there were no true roosts involved in the cage operation of Ranch "A," the narrow wooden supports under the wire floors were treated as roosts. They were sprayed with 3% and 1% sprays of malathion emulsion. A 3-gallon capacity hand pump sprayer equipped with a nozzle producing a coarse droplet spray was utilized. The nozzle was held just above the surface to be treated, applying the spray to the point of run-off on the top and sides of the "roosts." Approximately 4 gallons of spray were applied per 1,000 lineal feet of roost area. The effectiveness of the spray program was tested at weekly intervals by examination of the marked birds in each cage. In a few instances either the marked birds or the bands were missing; in these cases random sampling of birds from the cage was conducted.

The residual effectiveness of the roost treatment was challenged at weekly intervals, beginning 2 weeks after treatment, by introducing active lice on marked birds at the rate of 50 to more than 200 per bird. At Ranch "B" the same general technique of application was used as at "A," applying 3% malathion emulsion spray to roosts at the rate of 2.5 gallons per 1,000 lineal feet, and 1% malathion spray at the rate of approximately 3.5 gallons per 1,000 lineal feet.

A 3% malathion roost spray at ranch "A" gave apparently complete control of the chicken body louse within 1 week. Birds in treated cages remained clean of lice for the 9-week period of observation except where exposed to artificial reinfestation. It appears that residual control effects were lost between 3 and 5 weeks after treatment, although lice failed to establish themselves in birds of one pen when introduced 5 weeks after roost treatment. Infested birds in untreated pens on ranch "A" maintained high levels of infestation during the month after treatment of the test pens. Treatment of roosts at ranch "A" with 1% malathion emulsion spray also resulted in an apparently complete kill of lice on poultry. Artificial reinfestation of birds was unsuccessful at 2 and 3 weeks following roost treatment, but at 4 weeks' post-treatment, some lice were able to survive. At ranch "B" effective control of lice was obtained with a 3% malathion emulsion roost spray but not with a 1% spray.

The apparent discrepancy between effectiveness of louse control at the two ranches using a 1% malathion emulsion roost spray is probably due to the fact that birds at ranch "A" were in close proximity to treated surfaces for 24 hours a day, whereas at ranch "B" the proximity to treated roost surfaces was limited to the hours of darkness alone. In either type of housing a 3% malathion emulsion spray provided apparently complete kill of the chicken body louse.

MALATHION IN THE CONTROL OF THE FOWL TICK.—*Laboratory tests.*—Some tests were performed prior to conducting field experiments on the efficacy of malathion in controlling the fowl tick. Twenty adult and nymphal ticks, some recently fed and others unfed, were placed in an open petri dish on a piece of filter paper which had been freshly sprayed with a 3% malathion emulsion to the point of run-off. It was found that all ticks held on the treated surface for 72 hours succumbed. Similar findings were obtained when a 1% malathion spray was used with a comparable number of ticks.

Further laboratory tests were conducted to determine duration of effectiveness of a treated surface in controlling the fowl tick. The interior of an empty, 100-capacity microscope slide box was sprayed with a 3% malathion emulsion to the point of run-off. Hiding places for ticks had been arranged in the box and a screened window provided for ventilation. At intervals following treatment, 20 to 65 ticks (nymphs and adults) were placed in the box. All of the ticks introduced, from shortly after spraying the box to 1 week later, were dead or moribund within 4 days of continuous exposure. Twenty ticks, placed in the box 19 days after its treatment, were, for the most part, dead or moribund within a week of continuous exposure. However, when this box was torn apart 7 weeks later, two normal ticks were found. They were survivors of the introduction at 19 days following treatment. These ticks were wedged in tightly beneath a wooden support in the box and were apparently protected from the malathion residue which killed all other ticks exposed at the same time. Presumably, these ticks survived because they did not remain in direct contact with the treated surface for any appreciable time.

To determine the effect of aging of a treated surface and of length of exposure periods of ticks to such a surface upon the mortality rates of ticks, the following tests were conducted. Ticks were placed on a piece of filter paper which had been sprayed with a 3% malathion emulsion to the point of run-off. The ticks were removed from the treated surface after having been exposed for varying periods of time and observed for a period of 2 to 5 weeks thereafter. Unexposed controls were held for similar periods and exhibited negligible mortality. Additional ticks were exposed at weekly intervals over a 4-week period using the same treated surface.

Results of these tests are given in table 1. From these data it is seen that up to 9-hours' exposure to a freshly treated surface was necessary to obtain 100% kill of ticks. The percentage kill was proportional to the length of exposure time. With aging of the treated surface, an increasing time of exposure was necessary to ensure 100% kill of ticks; at 2 to 4 weeks following treatment of the surface 34 hours exposure was necessary to obtain 100% kill. Thus it seems possible that ticks might migrate into a

Table 1.—Residual effectiveness of 3% malathion spray treated surface against *Argas persicus*.[a]

Days After Treatment of Surface	Per Cent of Ticks Moribund or Dead[b]					
	Hours of Exposure to Treated Surface					
	1	3	5	9	12	24
0	85	94	95	100	100	100
7	31	46	46	46	72	100
14	52	25	34	38	48	84
21	6	31	35	56	62	72
28	8	33	50	—	75	91

[a] Eighteen to 27 ticks used per test.
[b] Morbidity and mortality recorded over a 2- to 5-week period following removal from treated surface.

Table 2.—Malathion and aldrin sprays against *Argas persicus* in chicken houses.

House No.[a]	Surface Treated (Sq. Ft.)	Spray per Sq. Ft. Surface Area (Ml.)	Infestation[b]											
			Weeks After Treatment											
			Pretreatment	1	2	3	4	5	6	7	8	10	13	15	20
1	1,300	9.7	100	0	—	0	0	1	0	0	1	1	—	—	0
2A	650	13.8	200	100	—	100									
2B	650	12.2	100	0	0	0	0	0	—	0					
3	4,000	8.7	600	200	200	150	150	—	200	150	—	—	—	150	
4	3,000	5.3	50	30	9	0	0	4	—	—	—	—	0		
5	700	11.4	125	75	70	75	65	75	—	—	—	—	36		
6	2,150	5.8	100	75	85	80	—	—	—	—	60				
7	2,150	6.1	400	2	16	1	—	—	—	—	0				

[a] Nos. 1 to 6 inclusive sprayed with 3% malathion; no. 7, with 2% aldrin.
[b] Ticks found per man in ½ hour. Heavier infestation collection figures expressed only in multiples of 25.

malathion treated area, obtain a blood meal, and escape to any nearby untreated area without being fatally affected, even shortly after spraying procedures had been completed.

FIELD TESTS.—Control of the fowl tick was attempted during the summer of 1955 in the vicinity of Davis, Yolo County, California. Seven farm yard type chicken houses were treated, 6 with a 3% malathion emulsion spray and 1 with a 2% aldrin spray. Aldrin was chosen for comparative purposes since Edgar *et al.* (1953) reported effective control of this tick with this insecticide. Weekly examinations of poultry houses were made to determine the effectiveness of treatment.

Prior to spraying each house, an estimate was made of the degree of infestation. Because of the discontinuous distribution of ticks, reliance was placed primarily on frequent observation to evaluate effectiveness of treatment. However, for comparative purposes, a count was made of all normal ticks found in each house during the weekly examinations and expressed as the number of ticks found per man in ½ hour (see Table 2). Malathion emulsion sprays were prepared as described above for louse sprays. Aldrin emulsion spray was prepared from a commercial product *Aldrex*.[3] Insecticides were applied with a pressure cylinder hand sprayer in such a manner that all exposed surfaces were wetted to the point of run-off. Particular attention was given to cracks and crevices likely to harbor ticks. The amount of spray used varied from 5.3 to 13.8 ml. per square foot of surface area, reflecting the wide range of absorptive qualities of surfaces treated.

Chicken House No. 1.—The malathion spray killed the greatest majority of ticks in this moderately infested house, but a few normal ticks were found in subsequent examinations. These were usually in deep crevices where they were apparently protected from the spray. Residual effects were noted 4 weeks following treatment, but thereafter an occasional tick was seen on a treated surface, apparently unaffected. This house also had an initial heavy infestation with the chicken mite. The treatment eradicated the infestation; no further mites appeared in the house during the 20-week period of observation.

Chicken House No. 2.—The initial treatment did not control the infestation; a large number of normal, adult ticks with newly laid eggs were found under several tight-fitting boards 1 and 3 weeks after initial treatment. The house was sprayed again, 3 weeks after the first treatment, with an effort being made to pry up as many hiding places as could be found prior to spraying. This resulted in the complete control of fowl ticks, no live ticks being found thereafter for the 8 weeks of observation. (See 2B in table 2).

Chicken House No. 3.—There were a great many hiding places in this house. Table 2 indicates that the infestation was not controlled by a single treatment. The weekly post-treatment examinations always exposed large numbers of normal adult and seed ticks whenever an effort was made to pry apart tightly fitting boards. Only those ticks in the more exposed recesses about the roosts seemed to have been affected by the malathion.

Chicken House No. 4.—Because of the light infestation it was not possible to adequately evaluate control in this house. No normal ticks were found during the third and fourth week post-treatment examinations, but on the fifth week, a few, freshly engorged adult ticks were seen. These may have been recently introduced or they may have been missed in earlier examinations. However, they were found on exposed surfaces and were unaffected by the malathion residue.

Chicken House No. 5.—This house was largely constructed of old lumber, much of which was splintered and riddled with holes, which offered numerous protected hiding places for ticks. The malathion spray, applied at a rate of 11.4 ml. per square foot was not effective in controlling those ticks hidden in the deeper cracks. Apparently these ticks survived if they remained secluded during the first few weeks following treatment.

Chicken House No. 6.—Only the ticks in the more exposed areas were affected by the malathion spray. Numerous normal ticks were found in several sites into which the spray had apparently not penetrated, even though pains were taken to thoroughly treat these particular sites.

Chicken House No. 7.—This house had one wall contiguous to that of house No. 6. The infestation was considerably heavier than in house No. 6. Both houses were approximately the same size and offered a comparable number of hiding places for ticks. A 2% aldrin spray was applied in this house at about the same rate as the 3% malathion was applied in house No. 6. Comparatively

[3] Shell Chemical Corporation.

few normal ticks were found in this house during the observation period as contrasted with the number found in house No. 6 (see Table 2). Eight weeks after treatment, no live ticks could be found in a half-hour search, while in house No. 6, 60 normal ticks were observed in a comparable length of time.

From field and laboratory tests, it is apparent that a single 3% malathion emulsion spray, applied at a rate of 5.3 to 13.8 ml. per sq. ft. surface area, will not control the fowl tick unless the insecticide comes into intimate contact with the ticks for a sufficient length of time. Most of the chicken houses in these tests offered hiding places in which ticks were protected from the malathion and from which they probably could move for short periods of time and remain unaffected. These ticks would provide a nucleus from which the infestation could rapidly build up once the malathion residue had dissipated.

A 2% aldrin spray is apparently more effective in controlling the fowl tick than a 3% malathion spray.

SUMMARY.—Toxic levels of malathion emulsion dips were determined for 9-month-old chickens and 12- to 15-week-old turkeys. All birds survived dipping in 1% malathion although some chickens showed temporary abnormal symptoms. Two per cent malathion dip killed 1 of 4 turkeys dipped and caused temporary abnormality in 2 more of 4 birds treated. Four per cent dips killed all birds treated, including one mature goose. Dead or moribund turkeys autopsied following dipping showed little gross pathology attributable to the treatment.

The chicken body louse, *Menacanthus stramineus* (Nitz.), was controlled effectively in field tests by spraying roosts with a 3% emulsion of malathion. A 1% roost spray controlled lice on only one of two poultry ranches. Residual effectiveness of roost sprays as determined by artificial reinfestation was largely dissipated within 3 to 5 weeks.

Results of laboratory and field tests directed against the fowl tick, *Argas persicus* (Oken), indicate that a 3% malathion emulsion sprayed surface was only effective in killing all ticks if they remained on the treated surface for a period ranging from 9 hours immediately following treatment, up to 34 hours 2 to 4 weeks after treatment. A 2% aldrin house spray was more effective in controlling ticks than a 3% malathion spray.

The chicken mite, *Dermanyssus gallinae* (DeG.), was completely eradicated from a heavily infested house by a 3% malathion emulsion spray. This concentration was used in attempting control of *Argas persicus*, and was undoubtedly greater than necessary for control of the chicken mite.

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REFERENCES CITED

Dicke, R. J., G. J. Lugthart, and R. H. Jones. 1955. Control of maggots in turkey dung with malathion. Jour. Econ. Ent. **48**(3): 342–3.

Edgar, S. A., C. D. Little, and J. F. Herndon. 1953. Control of the fowl tick, *Argas persicus* (Oken), on an Alabama poultry farm. Amer. Vet. Med. Assoc. Jour. **123**: 446–7.

Furman, Deane P. 1953. Comparative evaluation of control procedures against the northern fowl mite. Jour. Econ. Ent. **46**(5): 822–6.

Furman, Deane P., Lloyd E. Vincent, and W. Stanley Coates. 1955. External parasites of poultry. Calif. Agr. **9**(1): 13.

Harding, Jr., W. C. 1955. Malathion to control the northern fowl mite. Jour. Econ. Ent. **48**(5): 605–6.

Moore III, Steve, and H. H. Schwardt. 1954. The control of external parasites of chickens in New York State. Poultry Sci. **33**(6): 1230–7.

Vincent, L. E., D. L. Lindgren, and H. E. Krohne. 1954. Toxicity of malathion to the northern fowl mite. Jour. Econ. Ent. **47**(5): 943–4.

The Crystalline Isomer of Allethrin as a Standard of Comparison for Fly Sprays[1]

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After the synthesis of allethrin in 1948 it was thought that this insecticide would serve as a welcome substitute for pyrethrins as a standard of comparison for house fly space sprays, and it has been so used in much work at the Beltsville, Md., laboratory. However, the finding of a related substance crystalline at room temperature, and so more suitable for that purpose, was still hoped for.

When the *α-dl-trans* isomer of allethrin was prepared (Schechter *et al.* 1951), the toxicity to house flies, *Musca domestica* L., of three pure samples of it, one a commercial product, was estimated in comparison with allethrin and pyrethrins by the turntable method (Gersdorff & Mitlin 1952). The isomer was found to be about one-third as toxic as allethrin and about eight- or nine-tenths as toxic as pyrethrins, and reasons were given suggesting that this compound might serve as the long-desired crystalline substitute. To gain information on its chemical stability as evidenced by bioassay and on the mathematical stability of the toxic relationships, the commercial sample and its stock kerosene solution, from which the sprays had been prepared for testing, were stored in the dark for subsequent comparisons with a freshly recrystallized sample and with allethrin and pyrethrins. This paper reports the results of such comparisons made after 12 months and again after 38 months together with a biometric study of them.

[1] Accepted for publication December 15, 1955.

MATERIALS AND METHODS.—The sample of *α-dl-trans* allethrin was the one prepared outside the Entomology Research Branch. It was selected for storage solely because it was available in abundant supply; no differences, either chemical or toxicological, had been found between it and the other two samples (*loc. cit.*). The sample of allethrin was of 94% purity as determined by the hydrogenolysis method. Two samples of pyrethrins were used, each was the complex contained in the complete extractives obtained from pyrethrum flowers without further processing for the removal of materials other than the toxicants. They had been proved to be biologically stable when held in kerosene stock solutions in the dark at room temperature and tested against house flies in comparison with allethrin both before and after the tests reported herein. However, in one sample 54% and in the other 61% of the total pyrethrins consisted of pyrethrin I and cinerin I as determined by the mercury-reduction method; the second sample, used in the final series of tests, has been found to be about one-eighth more toxic than the first, as would be expected (Gersdorff 1947).

The method of comparison required the determination of dosage-mortality curves. Mortality of house flies caused by refined kerosene sprays at selected concentrations was determined in replicated tests made with the Campbell turntable. Knockdown of flies was also estimated. Approximately 100 flies, averaging $2\frac{1}{2}$ to $3\frac{1}{2}$ days in age, were used in each test. All sprays in a series were tested simultaneously on each of six populations of flies. All flies were from the strain used in the previous studies with the crystalline isomer, the 1948 NAIDM strain, and were reared in the laboratory by the same procedure.

After 12 months' storage of the stock solution of the *α-dl-trans* allethrin, 16 mg. per milliliter (2%), aliquots of it diluted with kerosene were tested in a series with the sprays of both standards.

After storage of the stock solution for 38 months, diluted sprays were again prepared from it and a similar comparison was made. Sprays freshly prepared from the stored sample and a recently recrystallized sample of it were included in this series of tests. By this time the stored sample, originally colorless, had acquired a slight yellowish color. When its stock solution was prepared, a negligible amount, estimated at less than 0.1% and carrying the color, failed to go into solution.

To evaluate relative toxicity and determine the precision of the estimates, the mortality data were subjected to probit analysis as described by Finney (1952).

KNOCKDOWN AND MORTALITY RESULTS.—The knockdown and mortality data are summarized in table 1. No differences are shown in the knockdown effect at comparable concentrations for the four treatments of the isomer. The knockdown value of the isomer, although high, is appreciably less than that of allethrin and pyrethrins, but, within the limitations imposed by tests planned to study mortality rather than knockdown, the difference after such storage is no greater than it was before (Gersdorff & Mitlin 1952).

Mortalities at comparable concentrations for the four treatments of the isomer likewise do not show real differences among themselves but do differ from those of the previous study (Gersdorff & Mitlin 1952). Especially when these differences are considered together with those for the standards in the two studies, their meaning is better perceived after statistical study of regression lines.

Table 1.—Knockdown and mortality of house flies caused by differently treated samples of *alpha-dl-trans* allethrin, and by pyrethrins and allethrin standards, 6 replicates.

MATERIAL AND TREATMENT	CONC. (MG. PER DL.)	PER CENT: Knockdown in 25 Minutes	PER CENT: Mortality in 1 Day
	Series 1		
Isomer, solution stored 12 months	800	100	97.1
	400	100	61.2
	200	98.7	17.1
	100	87.2	3.3
Allethrin[a]	200	100	80.0
	100	100	46.0
	50	98.3	14.6
	25	89.1	2.2
Pyrethrins[a]	800	100	82.9
	400	100	56.5
	200	100	30.8
	100	100	12.1
	Series 2		
Isomer, Recrystallized sample[a]	800	100	95.0
	566	100	83.8
	400	100	60.8
	283	99.9	33.1
	200	99.2	13.0
	100	92.4	0.7
Sample stored 38 months	800	100	91.5
	566	100	82.0
	400	100	67.7
	283	100	35.3
	200	99.3	10.4
	100	95.9	1.8
Solution stored 38 months	800	100	90.5
	566	100	80.3
	400	100	61.2
	283	99.9	39.2
	200	99.6	16.3
	100	92.5	2.1
Allethrin[a]	200	100	90.2
	133	100	70.1
	88.9	100	57.2
	59.3	100	30.7
	39.5	100	7.6
	26.3	99.8	2.2
Pyrethrins[a]	759	100	93.4
	506	100	81.6
	338	100	67.7
	225	100	48.4
	150	100	29.9
	100	100	24.0

[a] Not stored.

EVALUATION OF RELATIVE TOXICITY.—Provisional regression lines were fitted graphically to the data in table 1. Since it appeared in series 2 that the data for the three treatments of the isomer could be fitted by parallel lines, the mean of the slopes of the three lines was obtained and used in the determination of provisional equations. From these equations the expected probits were determined for the treatments. In all other cases the expected probits were determined from the individual lines, the slopes being significantly different. In the subsequent analysis

of chi-squares in the generalization procedure for the three treatments, there was no evidence that the data could not be fitted by parallel lines; therefore, this procedure was carried to completion. To allow for heterogeneity between populations of flies, however, it was necessary to use a heterogeneity factor of 3.738 in the calculation of variances for these treatments. Other heterogeneity factors required were 7.697 for allethrin and 5.041 for pyrethrins in series 2, and in series 1, 10.064 for the isomer, but none for either allethrin or pyrethrins.

The weighted equations, showing the regression of mortality, expressed in probits, on concentration in milligrams per deciliter, expressed as logarithms, are given below, together with the standard errors of the regression coefficients (b).

		Standard Error of b
Series 1		
Isomer	$Y=4.116\ X-5.346$	0.43
Allethrin	$Y=3.158\ X-1.419$	.12
Pyrethrins	$Y=2.334\ X-0.867$	.09
Series 2		
Isomer:		
Recrystallized sample	$Y=4.192\ X-5.657$	.13
Stored sample	$Y=4.192\ X-5.651$	.13
Stored solution	$Y=4.192\ X-5.637$	.13
Allethrin	$Y=3.642\ X-2.067$	.28
Pyrethrins	$Y=2.518\ X-0.897$	.19

From these equations the median lethal concentrations (LC-50's) were calculated. Relative toxicities, calculated as the inverse ratios of the relevant pairs of LC-50's, together with their standard errors, are given in table 2.

Table 2.—Relative toxicities of differently treated samples of the α-*dl-trans* isomer of allethrin.

Material and Treatment	LC-50 (Mg. per Dl.)	Toxicity Relative to—	
		Allethrin	Pyrethrins
Series 1			
Isomer, solution stored 12 months	326.3±19.0	0.331±0.021	0.999±0.064
Allethrin	107.9± 2.6	1.0	3.022±0.114
Pyrethrins	326.1± 9.4	0.331±0.013	1.0
Series 2			
Isomer, recrystallized sample	348.7± 9.0	0.250±0.012	0.631±0.034
sample stored 38 months	347.4± 9.0	0.251±0.013	0.633±0.034
solution stored 38 months	344.9± 8.9	0.253±0.013	0.638±0.034
Allethrin	87.2± 3.7	1.0	2.524±0.160
Pyrethrins	220.0±10.3	0.396±0.025	1.0

DISCUSSION.—The statistical study of the results clearly demonstrates that the three samples of the isomer in series 2 do not differ in toxicity. Despite the slight discoloration of the crystalline sample after 38 months' storage, no loss in toxicity resulted. There was also no deterioration in the kerosene solution. Therefore, since one bioassay was of a recrystallized sample and an acceptable ratio of toxicity was obtained for the two standards, as compared with previous results, the results with these three samples as well as the sample used in series 1 may be treated as additional bioassays of the isomer. There are, then, differences in these three estimations of the relative toxicity of the isomer as compared with the estimation in series 1 and those in the previous study.

In series 2 all tests were made on flies slightly older than those used in the other comparisons, averaging about ½ day more, and the particle size was decreased from 12 to 10 microns in mean diameter of oil droplets of the settling mist, this decrease often being made in the turntable procedure to offset the effect of higher age on mortality. However, to suggest that these slight differences may be responsible for the lower ratio of toxicity obtained for the isomer in this series would require an explanation of the fact that the ratio of the toxicity of allethrin relative to pyrethrins was not affected proportionately, for this ratio is just what it should be according to the mean of many past determinations.

Altogether seven estimations have been made by this method, the four reported in this paper and three reported previously (Gersdorff & Mitlin 1952). Pertinent data and additional estimations have been assembled in table 3. All estimations of concentrations and ratios were made from the equations. In obtaining the general results the completely weighted statistical method was used, with allowance for the differences in number of bioassays. However, the derivation of simple arithmetic means or of partially weighted means would have given results for all measurements, including equations, of no practical difference. For example, as simple arithmetic means one would obtain for the general means of the LC-50's of the isomer and allethrin and of their ratio 310.0, 93.23, and 0.301, respectively. By any short approximate weighting scheme, such as for number of tests, the results would be even closer to those reported in table 3. In ratios involving pyrethrins no adjustment has been made to allow for the slightly higher toxicity of the sample of that standard used in series 2. The general estimations are the results of 448 tests with 45,440 flies representing 26 different populations, and it would appear that 0.29 as toxic as allethrin and 0.74 as toxic as pyrethrins are fair expressions of the relative toxicity of the isomer. However, these ratios are subject to relatively wide variation with different populations of flies, a range of 0.25 to 0.35 being obtained for the first ratio and 0.63 to 1.0 for the second. The range for the ratio of toxicity of the isomer to allethrin is about the same relatively as that (2.3 to 3.3) obtained for the ratio of toxicity of allethrin to pyrethrins in many similar comparisons with purified allethrin and stable pyrethrum extract.

An assumption that susceptibility to different toxicants will vary proportionately with different populations of flies accompanies a belief in a stable ratio. This assumption may be unwarranted, and disproportionate variation in this respect would lead to variations in toxic ratios. This effect could be materially increased by the variation due to slope in the comparison of two toxicants with regression lines of different slope, a comparison already restricted to approximation because of the variation of the ratio at different levels of mortality with the same populations. It is therefore suggested that the standard chosen for a comparison should not have a regression coefficient greatly different from that of the compound under bioassay. That this would be advantageous has been foreseen in the past; in previous studies by this method compounds have been compared whenever possible with a standard similar in chemical structure with the expectation that such compounds would be more likely to give equations with nearly the same regression coefficient than are compounds of very dissimilar structure.

Table 3.—Estimations of the relative toxicities of allethrin, its crystalline isomer, and pyrethrins.

Estimation Number	Number of Tests (Concentrations × Replicates)	Approximate Number of Flies per Material	LC-50 (Mg. per Dl.)			Toxicity of Isomer Relative to—						Toxicity to Allethrin Relative to Pyrethrins Based on		
						Allethrin Based on			Pyrethrins Based on					
			Isomer	Allethrin	Pyrethrins	LC-5	LC-50	LC-95	LC-5	LC-50	LC-95	LC-5	LC-50	LC-95
From Gersdorff & Mitlin (1952):														
1	4×7	2,710	266.5	82.94	254.0	0.233	0.311	0.415	0.538	0.953	1.69	2.31	3.06	4.07
2	4×7	2,560	268.4	94.91	219.5	.250	.354	.501	.572	.818	1.17	2.28	2.31	2.34
3	4×7	2,590	267.8	—[a]	—[a]	.251	.354	.502	.573	.820	1.17	—	—	—
Series 1:														
4	4×6	2,270	326.3	107.90	326.1	.250	.331	.473	.495	.999	2.02	1.98	3.02	4.62
Series 2:														
5	6×6	4,060	348.7	87.16	220.0	.218	.250	.286	.346	.631	1.15	1.59	2.52	4.02
6	6×6	4,010	347.4	—[a]	—[a]	.219	.251	.288	.347	.633	1.15	—	—	—
7	6×6	4,040	344.9	—[a]	—[a]	.220	.253	.290	.350	.638	1.16	—	—	—
All	—[b]	—[b]	314.8 ±5.8	90.1 ±1.9	233.0 ±6.3	.230	.286 ±0.008	.356	.429	.740 ±0.024	1.28	1.87	2.59 ±0.09	3.59

Equations in comparison	Number	Isomer	Allethrin	Pyrethrins
	1	Y=3.722 X−4.030	Y=2.902 X−0.568	Y=2.384 X−0.734
	2	Y=3.241 X−2.871	Y=2.499 X+0.058	Y=2.479 X−0.805
	3	Y=3.241 X−2.868	same	same
	All 7	Y=3.820 X−4.542	Y=3.132 X−1.121	Y=2.466 X−0.838

[a] Comparison is to be made with LC-50's next above, these having been determined but once in each series.
[b] Total number of tests and flies: For the isomer, 216 and 22,240; for each of the other materials, 116 and 11,600.

Generally the slope of the isomer line bears about the same relationship to the slope of the allethrin line as the latter does to the slope of the pyrethrins line. The close approach of the allethrin line to parallelism with the pyrethrins line in comparison 2 is very unusual in studies of these two materials by this method.

To show the extent to which the ratio may vary between the extremes of mortality level in a single comparison of such curves, it was determined at the 5 and 95% levels from the equations for each comparison and is also given in table 3. From the ratios determined from the general equations it may be seen that the range between the extremes of mortality levels is wider than is that based on LC-50's, this being so for the three types of ratio, the isomer compared with each standard, and allethrin compared with pyrethrins. Also, the seven comparisons are within the range between extreme ratios, well within with the second and third types of ratio but nearer the extremes with the ratio isomer to allethrin. To see in another way the extent of the variation of the latter, the mortality levels required in comparisons 1, 2, 4, and 5 to give 0.286, the mean relative toxicity of the isomer as compared with allethrin, were calculated from the equations for the isomer and allethrin. They were, respectively, 31.6, 15.5, 19.6, and 94.8%. It may also be calculated that the ratios of toxicity of the isomer and allethrin as estimated from the LC-50's in the same four comparisons would be obtained from the general equations at mortality levels of 73.7, 94.6, 86.5, and 15.5%, respectively.

Except for the simpler chemical nature of the isomer with the accompanying assurance of high purity and uniformity and the greater ease of handling a crystalline material, the isomer, then, is no better than allethrin itself as a substitute for pyrethrins as a general standard of comparison. Discoloration of the isomer, although slight and unaccompanied by a demonstrable loss in toxicity, when stored in the dark for 38 months, indicates that some deterioration is possible.

SUMMARY.—Further evaluations were made of the relative toxicities of allethrin, its crystalline (*α-dl-trans*) isomer, and pyrethrins when applied as contact insecticides in refined kerosene on the Campbell turntable. The house fly, *Musca domestica* L., was used as the test insect.

A sample of the isomer stored in the dark for 38 months, although developing a slight yellow color, was as toxic as when recrystallized.

The sample stored as a 2% kerosene solution in the dark for 38 months suffered no loss in toxicity.

A comparison of seven estimations of the relative toxicity of the isomer by this method, the four in this study and three previous ones, showed that it was 0.29 as toxic as allethrin and 0.74 as toxic as pyrethrins. These ratios are subject to relatively wide variation with different populations of flies, a range of 0.25 to 0.35 being obtained for the first ratio and 0.63 to 1.00 for the second.

The knockdown value of the isomer, although high and showing no deterioration after 38 months' storage, was appreciably less than that of allethrin and pyrethrins.

The isomer has proved no better biometrically than a number of samples of purified allethrin as a substitute for pyrethrins as a standard of comparison in bioassays by this procedure.

REFERENCES CITED

Finney, D. J. 1952. Probit analysis. 318 pp. Cambridge.

Gersdorff, W. A. 1947. Toxicity to house flies of the pyrethrins and cinerins, and derivatives, in relation to chemical structure. Jour. Econ. Ent. **40**(6): 878–82.

Gersdorff, W. A., and Norman Mitlin. 1952. A bioassay of some stereoisomeric constituents of allethrin. Jour. Wash. Acad. Sci. **42**(10): 313–18.

Schechter, Milton S., F. B. LaForge, A. Zimmerli, and J. M. Thomas. 1951. Crystalline allethrin isomer. Jour. Amer. Chem. Soc. **73**: 3541–2.

Studies of the Nutrition of the Pink Bollworm Using Purified Casein Media[1]

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The nutritional requirements of insects can be best determined by the use of dietary constituents of known chemical composition. Furthermore, it is necessary to have an aseptic environment, since microorganisms are know to supply essential nutrients for many insects. A great deal is known about the general food requirements of insects reared aseptically (Trager 1953), but the phytophagous insects, particularly in the order Lepidoptera, have received little attention.

The pink bollworm, *Pectinophora gossypiella* (Saund.), has been successfully reared in our laboratory under aseptic conditions on synthetic media containing albumen as the protein (Vanderzant & Reiser 1955). Although normal moths were obtained, the growth rate and the number that pupated were lower than expected. Consequently, a new medium was developed in which albumen was replaced by casein. Larvae reared on this medium grew at a normal rate, and it has several other advantages over the albumen medium. In other studies with phytophagous insects, Beck *et al.*, (1949) used a casein medium for rearing the European corn borer, *Pyrausta nubilalis* (Hbn.), but the diet had to be supplemented with plant extracts. Using a modification of Beck's medium, without plant extracts, Ishii & Urushibara (1954) reared the rice stem borer, *Chilo simplex* (Butler).

The basic casein medium for rearing the pink bollworm and the results obtained with this medium and various modifications of it are described here.

EXPERIMENTAL PROCEDURE.—The procedures for preparing the aseptic eggs and vials were essentially the same as previously described (Vanderzant & Reiser 1956). The preparation of the casein medium is described below. The eggs were collected from oviposition boxes, washed with a sterilizing solution according to the method of Stride (1953), allowed to hatch, and the larvae transferred to screw-capped vials containing sterile media, one to each vial. Observations on the growth and development of the larvae were made at frequent intervals and growth rates recorded. A few larvae that died the first day owing to drowning, injury in handling, or similar factors were discarded. Vials contaminated with microorganisms were also discarded. Losses due to these factors were extremely small.

The average weights of the pupae were also determined. Males and females were not weighed separately even though they differ in weight because approximately equal numbers of both sexes were reared in each group. Upon emerging, moths were placed in oviposition boxes so that production and fertility of eggs could be observed.

PREPARATION OF BASIC MEDIUM.—The composition of the casein medium was based upon the albumen medium, although minor changes were made in the amounts of several constituents. Because a great deal of browning occurred when glucose was included in the medium, sucrose was used instead. Albumen coagulated on heating, to form a solid medium, but it was found necessary to add agar to the casein medium to form a firm gel upon which the larvae could feed; otherwise, the mixture was wet and sticky and the larvae grew slowly. Cystine and glycine were added because casein is deficient in these amino acids. The ingredients of the basic casein medium per 100 grams are as follows:

	Grams
Casein, "vitamin free" (Nutritional Biochemicals Co.)	5.0
L-cystine (Nutritional Biochemicals Co.)	0.10
Glycine (Dow N.F.)	0.15
Sucrose, C.P.	8.0
Corn oil (Mazola)	1.0
Cholesterol, U.S.P.	0.3
Salt mixture "W" (Wesson's, Nutritional Biochemicals Co.)	0.7
Choline chloride	0.1
Cellulose (Whatman ashless powder, standard grade)	4.0
Agar (Difco Co.)	3.0
Sodium alginate (Kelcosol, Kelco Co.)	0.5
Vitamin solution (Vanderzant and Reiser 1956)	1.0 ml.
2*M* potassium hydroxide to pH 6.5	
Water to 100 grams	

The choline chloride was added as a solution of 0.1 gram per milliliter of water. The cholesterol was ground intimately with sucrose in a mortar in the proportions used for the medium, and this mixture was weighed out in the amounts needed. This method produced finely divided particles of cholesterol which could be dispersed homogeneously through the medium. The other constituents were weighed out as needed.

Casein, cystine, and glycine were mixed with one-third of the water and enough 2*M* potassium hydroxide was added to dissolve the casein and bring the pH to approximately 7.0. Agar was dissolved in one-third of the water over a boiling-water bath. The remaining ingredients were mixed together, and then added to the casein mixture in a Waring Blendor. The acidity was adjusted to pH 6.5 with 2*M* potassium hydroxide if necessary. The agar solution was finally added and the medium heated in the boiling-water bath until fluid in consistency. After cooling, 1.5- to 2.0-gram pieces of medium were cut out, placed in screw-capped vials, and sterilized in an autoclave for 20 minutes at 15 pounds' pressure. The inoculation of vials and observations were made as previously described.

Modifications of this medium were prepared in the same manner, the substances studied being either added or omitted.

RESULTS WITH BASIC MEDIUM.—Larvae developed more rapidly in the casein medium than in the albumen medium. This acceleration in the growth rate indicates that the casein medium provided either a better nutrient content or a superior rearing environment, or both. The main difference in nutrient value between the two media was in the protein. Although albumen has a more ade-

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quate amino acid content, casein supplemented with cystine and glycine gave better results. Even without these two amino acids it was possible to rear normal pink bollworms, (see Table 2).

The physical properties of the medium, such as hardness and texture, may also have contributed to the improved results. Agar, which has no nutritive value, produced a firm gel in the casein medium whereas the coagulable property of the protein alone thickened the albumen medium. However, it is unlikely that the physical properties of the medium could have produced such a marked effect upon the growth of the larvae. In the experiments performed in developing the albumen medium, wide variations in the water, fat, sugar, and albumen content, which changed the physical consistency markedly, failed to change the growth rate of the larvae.

On the casein medium and modifications of it, pupation began as early as the 14th day, reached a maximum between 20 and 25 days, and had practically ceased by the 30th day. Pupation of larvae reared on all albumen media was slow, usually beginning about the 28th day and extending over so long a period that there was no sharp dividing point between resting and nonresting larvae.

Typical data for pupation rates and pupal weights on casein media are given in table 1. These three experiments were chosen because there were large numbers of resting larvae and it was possible to obtain data for comparison with the nonresting larvae. Pupation ranged from 52 to 76% within 30 days. Between 30 and 60 days less than 8% pupated. After 60 days the remaining resting larvae were covered with wet cotton in a petri dish and pupae formed within 2 weeks. Pupae from resting larvae were much larger than those from nonresting larvae, regardless of the modification of the medium. This was expected because insects that survive a long period in the diapause would probably have a large amount of food reserves, as reflected in their weight, whereas small larvae would not be as likely to survive.

A larval period of 14 to 30 days is normal for the pink bollworm (Fenton & Owen 1932). Since larvae reared on casein media matured at a comparable rate (see tables), the pupation rate was chosen as a reliable means of evaluating various modifications of the basic casein medium. Methods of growth evaluation, such as determination of the larval instar at any particular time and width of head capsule, are not satisfactory for the pink bollworm because the larvae usually feed inside the medium where they cannot be seen. After the feeding period they emerge and pupate at the top of the vial, on the surface of the medium, or next to the vial wall.

Another method used for comparison of the media was the determination of weights of 2- or 3-day-old pupae. On the best albumen medium pupal weights averaged about 12 mg. Since this is somewhat lower than the average weight of field specimens, an increase in the pupal weight should indicate which modifications improved the casein medium.

Egg production was estimated for the basic medium only. One group of 14 moths, 6 males and 8 females, produced approximately 579 fertile eggs, or 72 eggs per female. A second generation of moths was also obtained on this medium. No further records were kept of oviposition, but fertile eggs were obtained from moths reared on the other casein media.

EFFECT OF MODIFICATIONS OF THE BASIC MEDIUM.—To determine whether any of the nutrients of the casein medium were present in growth-limiting amounts, a series of experiments were performed to test the effects of variation in amounts and omission of some of the nutrients. As shown in table 2, no significant gain in weight or in pupation rate resulted from increasing the amount of vitamins or adding yeast extract or nucleic acid and thymine to the basic medium. It was concluded, therefore, that the initial vitamin concentration was sufficient for normal growth of the larvae and that no other growth factors were provided by the yeast extract, nucleic acid, and thymine. The results with nucleic acid confirm the earlier findings with the albumen medium (Vanderzant & Reiser 1956).

When the casein content was increased, the results were not appreciably different from those obtained with the basic medium. Omission of cystine and glycine caused a marked delay in pupation, only 23% pupating in 30 days, and a rise in the number of resting larvae. This effect was confirmed in a later experiment with a modified medium containing 0.25 gram of fat, where omission of cystine and glycine resulted in 61% pupation as compared with 80% in the control. Moreover, a number of abnormal pupae and moths were obtained when cystine and glycine were omitted. It appears that either cystine or glycine or both have a beneficial effect or are necessary for pupation. Both these amino acids have been reported to be essential for the growth of other insects, and cystine in particular was found to take some part in the molting process of *Lucilia sericata* (Meig.) (Michelbacher *et al.* 1932), *Drosophila melanogaster* (Meig.) (Lafon 1939),

Table 1.—Typical pupation rates and pupal weights of pink bollworms on three nutritionally adequate media.

Changes in Basic Medium	Number of Larvae	Pupae from Nonresting Larvae (within 30 Days)		Pupae from Resting Larvae		
					After 60 Days	
		Number	Average Weight (Milligrams)	Number Between 30 and 60 days	Number Weighed	Average Weight (Milligrams)
None	54	41	11.6	2	9	18.3
Cholesterol 0.1 gram	27	14	13.0	2	10	20.0
Ergosterol 0.3 gram	54	29	13.9	4	19	20.1

Table 2.—Effect of the addition or omission of various nutrients upon the growth of the pink bollworm.

Nutrient Added or Omitted per 100 Grams of Basic Medium	Number of Larvae	First Pupa (Days)	Per Cent Pupating Within 30 Days	Average Weight of Pupae (Milligrams)
No modification	54	18	66	11.6
Added:				
Vitamin solution 0.5 ml.	58	20	67	10.8
Yeast extract 1.0 gram	38	18	61	12.1
Thymine 0.1 gram				
Nucleic acid 1.0 gram	44	21	64	13.1
Casein 1.0 gram	44	20	80	12.9
Omitted:				
Cystine and glycine	112	21	23	12.0
Choline chloride	32	—	—	—

Aedes aegypti (L.) (Goldberg & deMeillon 1948), and *Blattella germanica* (L.) (House 1949). Their exact role in the growth and development of the pink bollworm is being studied further.

The omission of choline chloride prevented the development of 28 larvae beyond the second instar and 4 beyond the third instar. On the 16th day 21 larvae were alive and still in the second instar. At 30 days 11 were alive and 4 of them were in the third instar. At 40 days no further development was noted and only 5 larvae were still alive.

Studies on other vitamin deficiencies were not made. Although casein is essentially free of vitamins, it contains traces of the vitamins that were added to the media, perhaps some of them in sufficient amounts so that an accurate effect of their deficiencies upon growth and development cannot be determined.

Previous experiments with albumen media on the effect of fat content on the size, rate of development, and pupation of the pink bollworm failed to show any differences between the fat concentrations that were studied. Because of the normal growth rate obtained on the casein medium, it was expected that a more critical evaluation of the effect of fat content could be made. Figure 1 shows the results obtained with large numbers of larvae reared on three media containing different amounts of corn oil. With 2 grams per 100 grams of medium, pupation was only 51% complete in 30 days; with 1 gram, 64%, and with 0.25 gram, 80%. It appeared, therefore, that an increase in fat content of the diet delayed pupation, thus producing more resting larvae and, as shown in table 1, an increase in average pupal weights. With 0.25 gram of fat per 100 grams medium pupation began as early as 14 days after inoculation.

The omission of fat had a marked effect upon the development of the pink bollworm. In three experiments the larvae pupated but moths did not emerge. In some cases death occurred early in the pupal period, but other insects lived until emergence and the pupal cases were cracked open by the moths attempting to emerge.

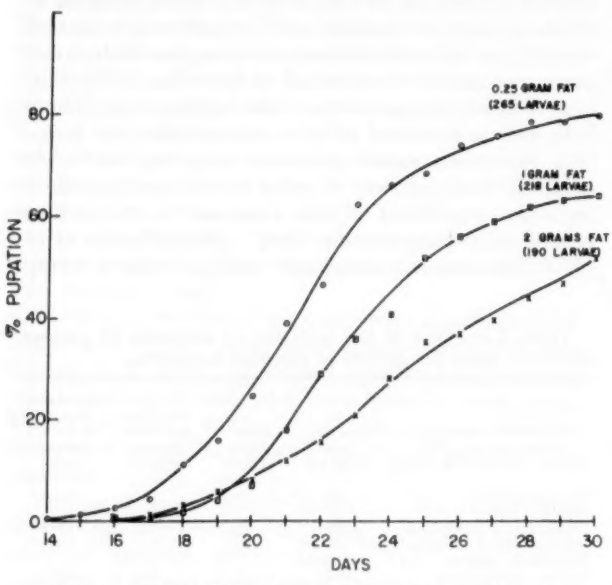


FIG. 1.—Effect of three levels of fat on the pupation of the pink bollworm.

The effect of the corn oil upon growth is most likely due to the essential fatty acid, linoleic acid, which makes up about 58% of commercial Mazola oil (Baldwin & Sniegowski 1951).

Fraenkel & Blewett (1946, 1947) demonstrated the need of *Ephestia* caterpillars for essential fatty acid. The caterpillars pupated but moths did not emerge. With graded increases in the amount of fatty acid emergence increased, but the moths were partially deficient in wing scales until the optimum amount was included in the diet. Such a marked dietary requirement for fatty acid has not previously been demonstrated in any other insect. The pink bollworm seems to have a similar need. However, Ishii & Urushibara (1954) could not find any fat requirement for the phytophagous rice stem borer, *Chilo simplex* Butler. The fat requirements of other moths have not been studied.

Because of the increased growth rate obtained with low levels of fat, in all subsequent experiments the amount of corn oil was decreased from 1 gram to 0.25 gram per 100 grams of medium.

The cholesterol requirement of the pink bollworm was studied using different amounts of cholesterol and the effect of the omission of cholesterol was also determined (Table 3). From 0.01 to 0.30 gram of cholesterol per 100 grams of medium were equally effective in promoting growth. The minimum level of cholesterol which would support normal development was not determined. However, in the absence of cholesterol the larvae grew slowly and either failed to pupate or formed abnormal pupae from which no moths emerged. These studies were conducted on a medium containing 0.25 gram of corn oil per 100 grams. Since Mazola oil contains approximately 1.5 per cent of sterols (Reiners 1955), there was approximately 3.75 mg. of sterols per 100 grams of medium. This is obviously not enough sterol to permit the normal development of the pink bollworm in the absence of cholesterol.

Normal moths were also reared on media containing the plant sterols ergosterol, sitosterol, or stigmasterol. Preliminary data indicated that larvae grown on these media were larger and had a slightly higher growth rate

Table 3.—Effect of various amounts of sterols upon growth of the pink bollworm.

Grams per 100 Grams of Medium		Number of Larvae	Per Cent Pupation		Average Weight of Pupae (Milligrams)
Sterol	Corn oil		20 Days	30 Days	
Cholesterol					
0.30	0.25	41	22	76	12.0
.10	.25	46	17	78	12.8
.05	.25	57	28	84	12.5
.01	.25	46	30	81	12.8
0	.25	40	0	23	—
Ergosterol					
0.30	1.0	54	20	54	13.9

than those reared on a medium containing cholesterol. Data for 0.3 gram of ergosterol in a medium containing 1 gram of corn oil are given in table 3. Although there were a large number of resting larvae in this experiment, the pupae from nonresting larvae were heavier than those obtained from media containing cholesterol. Plant sterols might be expected to have a greater nutritive value than cholesterol because they occur in the natural food of the pink bollworm.

Since very little is known about the mineral requirements of insects (Trager 1953), the content of Wesson's salts for the albumen medium was arbitrarily chosen as 1 gram per 100 grams of medium. When albumen was replaced by casein, these salts were reduced to 0.7 gram because an additional amount of salt was formed when the casein was dissolved in the potassium hydroxide solution. Traces of inorganic salts were also present in the other constituents.

Studies were made of the effect on growth of varying the content of Wesson's salts (Table 4). The other substances contributing to the inorganic salt concentration were constant. When the salt content was increased from 0.35 gram to 1.75 grams per 100 grams of medium, a marked effect on the growth rate was observed. Larvae pupated more rapidly, the most marked effect occurring 20 days after inoculation. At 1.75 grams of Wesson's salts per 100 grams, pupation was complete. The largest larvae, 14.9 mg., were reared on a medium containing 1.2 grams. From these data it appears that the initial content of these salts, 0.7 gram per 100 grams of basic medium, was too low for maximum growth.

The effect on growth of decreasing the sucrose content of the medum is also shown in table 4. A comparison between concentrations of 8, 6, and 4 grams of sucrose per 100 grams of medium containing 0.7 gram of Wesson's salts indicated that decreasing the sucrose content had the same effect upon growth rate and size as increasing the salt content. If these two effects are cumulative, they should produce a higher pupation rate and pupal size than was obtained from previous experiments. This was actually found to be the case with a medium containing 4 grams of sucrose and 1.2 grams of Wesson's salts per 100 grams.

It should be pointed out that any change in the amount of protein, fat, and carbohydrate will change the caloric level of the medium. Further studies on the interrelationships of various nutrients should reveal the reasons for the results of these experiments.

Table 4.—**Effect of various amounts of sucrose and Wesson's salts on the growth of the pink bollworm.**

Grams per 100 Grams of Medium		Number of Larvae	Per Cent Pupation			Average Weight of Pupae (Milligrams)
Sucrose	Wesson's Salts		15 Days	20 Days	30 Days	
8	0.35	28	0	3.6	71	13.0
	.70[a]	46	4	17	78	12.2
	1.20	56	11	71	84	14.9
	1.75	36	6	75	100	13.7
6	.70	59	3	75	86	14.0
4	.70	45	4	73	93	14.1
	1.20	52	12	89	94	15.2

[a] Basic medium.

SUMMARY.—A purified casein medium has been prepared which will permit the rearing of pink bollworms, *Pectinophora gossypiella* (Saund.) in the normal period of time. Normal moths that deposited fertile eggs were obtained.

Variations were made in the nutrient content of the medium in an attempt to produce larger and more rapidly developing larvae and to reduce the number of resting larvae.

As the fat content was reduced from 2 grams to 0.25 gram per 100 grams of medium, the pupation rate increased with a concomitant decrease in the number of resting larvae. Omission of cystine and glycine increased the number of resting larvae.

An increase in the vitamin content or the addition of yeast extract, nucleic acid, and thymine had no effect upon growth rate or size of the larvae.

The omission of fat and choline prevented larval development. When cholesterol was omitted, many larvae pupated, but the pupae were abnormal. Media containing from 0.01 gram to 0.30 gram of cholesterol per 100 grams produced larvae of similar size with a similar rate of development. Cholesterol could be replaced by ergosterol, sitosterol, or stigmasterol.

Larval size was increased and the growth and pupation rates accelerated when either the sucrose content was reduced or the Wesson's salts content was increased, or both. The optimum content of sucrose was found to lie between 4 and 6 grams and Wesson's salts between 1.20 and 1.75 grams per 100 grams of medium.

REFERENCES CITED

Baldwin, A. R., and M. S. Sniegowski. 1951. Fatty acid compositions of lipids from corn and grain sorghum kernels. Jour. Amer. Oil Chem. Soc. **28:** 24–7.

Beck, S. D., J. H. Lilly, and J. F. Stauffer. 1949. Nutrition of European corn borer, *Pyrausta nubilalis* (Hbn.). I. Development of a satisfactory purified diet for larval growth. Ann. Ent. Soc. Amer. **42:** 483–96.

Fenton, F. A., and W. L. Owen, Jr. 1932. The pink bollworm of cotton in Texas. Tex. Agr. Expt. Sta. Misc. Pub. 100.

Fraenkel, G., and M. Blewett. 1946. Linoleic acid, vitamin E and other fat-soluble substances in the nutrition of certain insects (*Ephestia kuehniella, E. elutella, E. cautella,* and *Plodia interpunctella.* (Lep.)). Jour. Exptl. Biol. **22:** 172–90.

Fraenkel, G., and M. Blewett. 1947. Linoleic and arachidonic acids in the metabolism of two insects, *Ephestia kuehniella* (Lep.) and *Tenebrio molitor* (Col.). Biochem. Jour. **41:** 475–8.

Goldberg, L., and B. De Meillon. 1948. The nutrition of the larva of *Aedes aegypti* Linnaeus. 4. Protein and amino acid requirements. Biochem. Jour. **43:** 479–87.

House, H. L. 1949. Nutritional studies with *Blattella germanica* (L). reared under aseptic conditions. III. Five essential amino acids. Canad. Ent. **81:** 133–9.

Ishii, Shoziro, and Hisayuki Urushibara. 1954. On fat and water soluble growth factors required by the rice stem borer, *Chilo simplex* Butler. Natl. Inst. Agr. Sci. Bull., Ser. C, No. 4, pp. 109–33.

Lafon, M. 1939. Recherches sur quelques aspects du besoin qualitatif d'azote. Essai sur le besoin qualitatif d'azote chez un insecte: *Drosophila melanogaster*, Meig. Ann. Physiol. et Physicochim. Biol. **15**: 215–60.

Michelbacher, A. E., W. M. Hoskins, and W. B. Herms. 1932. The nutrition of flesh-fly larvae, *Lucilia sericata* Meig. Jour. Exptl. Zool. **64**: 109–28.

Reiners, R. A. 1955. Corn Products Refining Co., Argo, Ill. Private communication.

Stride, G. O. 1953. On the nutrition of *Carpophilus hemipterus*, L. Coleoptera: Nitidulidae. Roy. Ent. Soc. London, Trans. **104**: 171–94.

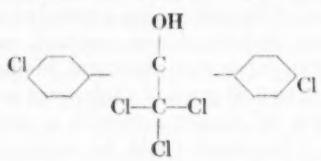
Trager, W. 1953. Chapter 14, Nutrition, pp. 350–86. *In* Insect Physiology, by K. D. Roeder. John Wiley and Sons, New York.

Vanderzant, Erma S., and Raymond Reiser. 1956. Aseptic rearing of the pink bollworm on synthetic media. Jour. Econ. Ent. **49**(1): 7–10.

Acaricidal Properties of Rohm & Haas FW-293[1]

JOHN S. BARKER[2] and F. B. MAUGHAN[2]

Rohm & Haas FW-293 was synthesized in 1953 and the chemical aspects characterized in a paper by Wilson & Barker (1954). The chemical structure is as follows:



In initial laboratory tests the compound displayed a high order of toxicity to the motile stages and eggs of two-spotted spider mites. Concentrations of 10 to 50 p.p.m. applied as a foliar spray gave a complete kill of all forms of this mite and provided long residual protection under laboratory conditions. Phytotoxicity tests with the compound at the concentration of 1 pound and 0.5 pound actual per 100 gallons indicated the compound was safe under greenhouse conditions on a variety of foliage such as beans, apples, peaches, and tomatoes.

Field studies conducted during the last 2 years have confirmed these laboratory observations and have proven the material an effective acaracide. The tests were conducted in various parts of the country with different formulations of the toxicant on apples, peaches, citrus, cucurbits, cotton, and ornamentals. Several species of mites were involved in these studies, such as citrus red mite (*Paratetranychus citri* (McGregor)), Pacific spider mite (*Tetranychus pacificus* (McG.), European red mite (*Metatetranychus ulmi* (Koch)), two-spotted spider mite (*Tetranychus telarius* (L.)), and the Schoenei spider mite (*Tetranychus schoenei* (McG.) 1955). The compound has been found generally non-toxic to most beneficial insects. The results presented in this paper are restricted to a series of tests conducted on the Rohm & Haas Experimental Farm on apples for the control of European red mites and two-spotted spider mites.

METHODS.—Four varieties of apples were employed, namely: McIntosh, Golden Delicious, Rome, and Stayman. The study was composed of six treatments plus an untreated check. They were replicated three times on each variety in a randomized block design. A pre-treatment count was made on a 50-spur-leaf sample selected at random around the tree. The mites on these leaves were removed by rotary brushes into an aqueous solution of *Triton X-100*[3] (iso-octyl phenyl ether of polyethylene glycol, 0.1% and ethanol, 20%). The resultant suspension was vacuum filtered leaving the mites evenly dispersed over a 14-cm. filter paper. The number of live mites occurring on five measured areas of the disc was counted with a binocular. From this figure the total number per 100 leaves calculated.

The applications were made on June 15. The initial kill determination was made with a binocular 24 hours later on a 50-leaf sample per tree. Following this observation, counts were made approximately every 2 weeks to determine residual control. These determinations were made in the same manner as the pre-treatment population.

RESULTS.—The data shown in table 1 give the pre-treatment population, initial kill, and the average number of live mites existing over a 40-day period following application. These latter figures are a mean of all replicates of all varieties based on the first three counts. The most effective treatments were the two highest dosages

Table 1.—Control of European red mite and two-spotted spider mite with various applications of Rohm & Haas FW-293 on Four Varieties of Apples,[a] Bristol, Pa.

FW-293 TREATMENT	PRE-TREATMENT[b] POPULATION	POPULATION[a,b] INDEX
EC—0.8 lb. actual	620	42.5
0.4	1522	76.6
0.2	750	140.8
WP—0.8 lb. actual	545	30.8
0.4	1275	48.3
0.2	585	230.8
Check	843	2537.2
L.S.D. at 5% level		81.5
at 1% level		108.4

[a] Average population existing over the 40-day period following treatment on June 15. This average is based on three counts made at 2-week intervals on three replicates of four varieties (McIntosh, Golden Delicious, Rome, and Stayman). Initial kill by all treatments was 100%.

[b] Expressed as live mites per 100 leaves consisting of both European red mites and two-spotted spider mites.

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[2] Rohm & Haas Co., Box 219, Bristol, Pa. The writers wish to acknowledge Dr. H. F. Wilson of Rohm & Haas Company who synthesized the compound and Dr. H. B. Haag of the Medical College of Virginia for the studies on mammalian toxicity.

[3] Trade mark—Rohm & Haas Co.

Table 2.—Population of mites[a] at varying intervals of time after treatment with Rohm & Haas FW-293,[b] Bristol, Pa.

Days After Initial Application	FW-293[c]			FE-293[d]			Check
	0.8	0.4	0.2	08.	0.4	0.2	
	Live Mites per 100 Leaves						
14	52.5	40.0	72.5	30.0	47.5	65.0	1660.0
28	35.0	127.5	107.5	32.5	35.0	160.0	3044.2
42	40.0	62.5	242.5	30.0	62.5	467.5	2312.5
L.S.D. at 5% level	247.8						
1% level	459.1						

[a] European red mites and two-spotted spider mites.
[b] Treatments replicated three times on McIntosh, Golden Delicious, Rome, and Stayman apples.
[c] Pounds actual per 100 gallons indicated below were applied as an emulsion concentrate (20%).
[d] Pounds actual per 100 gallons indicated below were applied as a wettable powder (25%).

of the emulsion concentrate and wettable powder. The least effective dosage was the 0.2 pound actual wettable powder which was significantly inferior to a similar dosage of the emulsion concentrate.

A further breakdown of these observations is shown in table 2. The separate interval counts are shown along with the L.S.D. values. It will be noted there was no significant build-up between 2 and 6 weeks at the two highest dosages of either the emulsion concentrate or wettable powder. At the dosage of 0.2 pound actual there was a significant build-up, particularly with the wettable powder under the conditions of this test.

Duration of Control.—An index of the season's performance of these treatments is given in table 3. This criterion is the duration of satisfactory control from the initial application made on June 15. Control was considered satisfactory if the population remained below five live mites per leaf. In this study the varieties are separated according to whether or not a dormant oil preceded the summer applications. With or without the dormant spray the highest dosage of the emulsion concentrate and wettable powder gave satisfactory control for 9 to 12 weeks (maximum possible under the conditions of the test). The same is true for the intermediate dosage of both formulations if a dormant spray preceded the summer applications. The other treatments (0.2 and 0.4 pound actual emulsion concentrate and wettable powder with no dormant oil and 0.2 pound actual emulsion concentrate with a dormant oil) were satisfactory for 6 to 8 weeks. The lower concentration gave longer protection when preceded by a dormant oil.

Table 3.—Control[a] of European red mite and two-spotted spider mite with one application of Rohm & Haas FW-293 made to apples on June 15,[b] Bristol, Pa.

FW-293 Concentration[c]	Prior Treatment	
	No Dormant Oil	Dormant Oil
	(Duration in weeks)	
0.8 EC	12.0	9.0
0.8 WP	11.0	9.0
0.4 EC	7.3	9.0
0.4 WP	6.4	9.0
0.2 EC	6.0	8.3
0.2 WP	6.0	7.4
L.S.D. at 5% level	0.8	1.0
at 1% level	1.1	1.4
Maximum protection possible	12.0	9.0

[a] Control was considered satisfactory as long as the population remained below five mites per leaf.
[b] Treatments replicated three times on McIntosh, Golden Delicious, Rome, and Stayman apples.
[c] Pounds actual per 100 gallons.

Number of Applications Required.—A third index of the season's performance is shown in table 4. This criterion is the number of applications required to give full season protection. Again control was considered satisfactory if the count remained below five live mites per leaf. When the population exceeded this base line on any tree, repeat applications were made. One application of the highest dosage of either the emulsion concentrate or wettable powder was sufficient for the season regardless of the dormant treatment.

With the lower dosages, the number of applications required was less when a dormant oil preceded the summer applications with a maximum of two sprays being required for any dosage in the absence of the dormant oil.

The performance of Rohm & Haas FW-293 in these tests is summarized as follows: (1) Regardless of the criterion used, that is, early build-up following initial application, or duration of control from one application or the number of applications required for full season control (less than five mites per leaf), FW-293 proved an effective acaricide.

Table 4.—Number of applications of Rohm & Haas FW-293 at various dosages required to give full season control[a] of European red mites and two-spotted spider mites on apple,[b] Bristol, Pa.

FW-293 Treatments[c]	Prior Treatment	
	No Dormant Oil	Dormant Oil
0.8 EC	1.0	1.0
0.8 WP	1.5	1.2
0.4 EC	2.0	1.2
0.4 WP	2.0	1.3
0.2 EC	2.0	1.5
0.2 WP	2.0	1.7
L.S.D. at 5% level	0.4	0.4
at 1% level	0.6	0.6

[a] Control was considered satisfactory as long as the population remained below five mites per leaf.
[b] Treatments replicated three times on McIntosh, Golden Delicious, Rome, and Stayman apples: Initial application made on June 15.
[c] Pounds actual per 100 gallons.

(2) Of these treatments, 0.2 pound actual of wettable powder was least effective. (3) At equal concentrations of actual compound the emulsion concentrate was more effective than the wettable powder. This was particularly true at the lowest dosage.

Effect of Timing Applications.—Data from a study conducted to determine the effect of timing on the performance of the compound are shown in table 5. These trees had no dormant oil prior to treatment. Three application sequences were chosen for this study, namely

Table 5.—Indices of acaricidal performance on Rohm & Haas FW-293[a] on Stayman apples on which the dates of application were varied.

Index	Applications: Only Early[b] 5/27	Both Early & Late[c] 5/27 & 6/29	Only Late[d] 6/29
I. Weeks of satisfactory control[e]			
Mean	13.9	9.0	7.3
Standard Error	0.50		1.29
II. No. of applications required for satisfactory control[e] (includes early and late applications plus additional application)			
Mean	2.0	2.0	1.1
Standard Error	0.1		0.03

[a] Four-tenths pound actual per 100 gallons EC.
[b] Maximum protection = 18 weeks (5/27–9/1).
[c] Maximum protection = 9 weeks (6/29–9/1).
[d] Maximum protection = 9 weeks (6/29–9/1).
[e] Less than 500 mites per 100 leaves.

an early application made on May 27, an early and late application made on May 27 and June 29, and a late application made on June 29. Additional sprayings were made as necessary to keep the population below five mites per leaf. The following points are obvious from this study: (1) One application of FW-293 at 0.4 pound actual (emulsion concentrate) on May 27 gave 14 weeks protection, or almost full season. (2) In the combination of early and late treatments (May 27 and June 29) the two applications of FW-293 gave full season control. (3) In the late treatment study the application of FW-293 on June 29 gave satisfactory control for the balance of the season.

MAMMALIAN TOXICITY.—Preliminary mammalian toxicity studies on FW-293 indicated the compound was relatively safe. In acute oral studies of albino rats, the LD_{50} for the technical material was found to be 0.73 $\pm$0.09 g/kg. Acute percutaneous investigations suggest a low order of toxicity, with an LD_{50} value for the emulsion concentrate on rabbits of greater than 10 cc/kg.

REFERENCES CITED

Wilson, H. F., and J. S. Barker, 1954. The acaracidal activity of alpha substituted benzhydrols. Unpublished paper presented before the American Chemical Society in 1954.

Asquith, D. 1955. Acaracide tests on apples in 1954. Jour. Econ. Ent. **48**: 329–30. and other unpublished data of the Research Division, Rohm and Haas Co., Philadelphia, Pa.

Maggots and Puparia in Stems and Seed Balls of Onions at Harvest[1]

D. G. FINLAYSON,[2] *Field Crop Insect Section, Entomology Laboratory, Kamloops, B. C.*

In 1953 at Kelowna, British Columbia, puparia of the onion maggot, *Hylemya antiqua* (Meig.), were taken from seed that had passed through all the mechanical processes of threshing and cleaning. This suggested another method by which the insect may become established on onions in new areas. Experiments were conducted in 1954 to determine the site of feeding, the incidence of the species, and the viability of purparia taken from seed balls before threshing and from seed after threshing and cleaning, both before and after diapause. In addition, the effect of treating the seed with DDT or dieldrin on emergence of flies was determined.

METHODS.—The investigation was conducted at Grand Forks, the centre of onion seed growing in British Columbia.

The sampling area consisted of 24 plots each containing 25 feet of row. No insecticide was applied at planting or during the growing season. Fermate for control of mildew was sprayed on the plants intermittently throughout the summer. Harvesting of the seed balls was completed in one cutting; the normal practice is to cut twice with a 1-week interval. The stems were cut at three different lengths to give the seed ball alone, with 1 inch of stem, and with 3 inches of stem.

Each of these three methods of cutting was replicated eight times. The seed balls of four replicates were picked into burlap sacks, which were dropped to the ground in the onion field, where contamination with soil-borne maggots was possible. The other four replicates, also picked into sacks, were not allowed to come into contact with the onion plants or the ground.

The seed balls were spread to dry on sheets of brown paper in a large building. Two replicates of each of the two types of picking and the three methods of cutting were examined before threshing. The stems and seed balls were broken open by hand to ensure that as many maggots and puparia as possible were obtained. A further examination was made after threshing and all remaining maggots and puparia were removed. The maggots and puparia were removed from the seed from the other two replicates after the seed balls had been threshed and the seed cleaned by mechanical processes.

One hundred puparia from each of the pre- and post-threshing examinations were placed in individual vials in a rearing cabinet at 75° F. and 70% R. H. to determine the percentage emergence. Puparia that did not produce flies in 30 days under the above conditions were placed in refrigeration for 90 days at 37° F. to break diapause, and then returned to the rearing cabinet. The viability of the puparia was calculated from the number of flies that emerged.

In addition to the above experiment, samples of 400

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[2] Associate Entomologist. The author is indebted to A. R. Brooks, Entomology Laboratory, Saskatoon, and W. R. M. Mason, J. F. McAlpine, and C. D. Miller, Entomology Division, Ottawa, for identifying the species.

gm. of seed (approximately 1 lb.) were taken from growers' seed after it had been freshly threshed and cleaned. The maggots and puparia in each sample were counted.

As a further study, puparia were placed in an evaporating dish with onion seed and treated with wettable 50% DDT or dieldrin, the normal seed treatment for onion maggot control in British Columbia (Finlayson & Handford 1954). The check was moistened with water to compensate for any effect the moisture added during seed treatment might have. They were planted in sterilized soil in lumite-covered battery jars to determine whether flies emerged from treated puparia. Water was added to the soil when necessary.

RESULTS AND DISCUSSION.—The numbers of maggots and puparia taken from the seed heads of the onions are shown in table 1. There appears to be no association between length of stem retained and numbers of maggots and puparia taken. There was a marked reduction in the numbers of maggots and puparia after the threshing and cleaning processes.

Table 1.—Average numbers of maggots and puparia, before and after threshing and cleaning, in onion seed harvested in various ways.

Method of Cutting Seed Heads	Before			After		
		Puparia			Puparia	
	Maggots	Whole	Broken	Maggots	Whole	Broken
Seed sacks in contact with ground						
Without stem	1.5	21.5	4.0	0.0	6.0	5.0
With 1-in. stem	4.5	62.0	22.5	0.5	5.0	3.5
With 3-in. stem	1.5	29.5	1.5	3.0	10.5	2.0
Seed sacks not in contact with ground						
Without stem	1.5	29.5	7.5	1.5	24.5	7.0
With 1-in. stem	9.0	48.0	3.0	0.5	20.5	14.0
With 3-in. stem	3.0	68.5	19.0	3.0	9.5	4.0

An additional 0.7 maggots, 1.3 whole puparia, and 0.6 broken puparia per plot were taken from seed from replicates examined by hand after cleaning.

The average numbers of maggots taken from 400-gm. samples of commercial onion seed were: *Hylemya* spp., 0.5; *Paragopsis strigatus* (Fall.), 11.9; *Fannia canicularis* (L.), 0.1; *Syrphus* sp., 1.2; and those of puparia: *Hylemya* spp., whole, 21.8; broken, 9.5.

Examination of the seed heads revealed that *Hylemya* spp. had fed and pupated in the enlarged terminal end of the stalk supporting the seed (Figures 1 and 2).

The species present in the seed heads and stems and commercial seed samples were: the onion maggot (*Hylemya antiqua*), the seed-corn maggot (*Hylemya cilicrura* (Rond.)), the onion bulb fly (*Paragopsis strigatus* (Fall.)), the little house fly, (*Fannia canicularis* (L.)), and *Syrphus* sp., presumably a predator of aphids or coccids.

The percentages of emergence from puparia taken from the seed heads before and after threshing respectively were: 28.0 and 18.0 without diapause and 33.0 and 18.8 after diapause. The difference between pre- and post-threshing samples was probably due to mechanical injury during threshing.

Small puparia that had been set aside for rearing as a possible sixth dipterous species produced a braconid

FIG. 1.—Terminal end of onion stalk showing gallery where maggot fed and pupated.

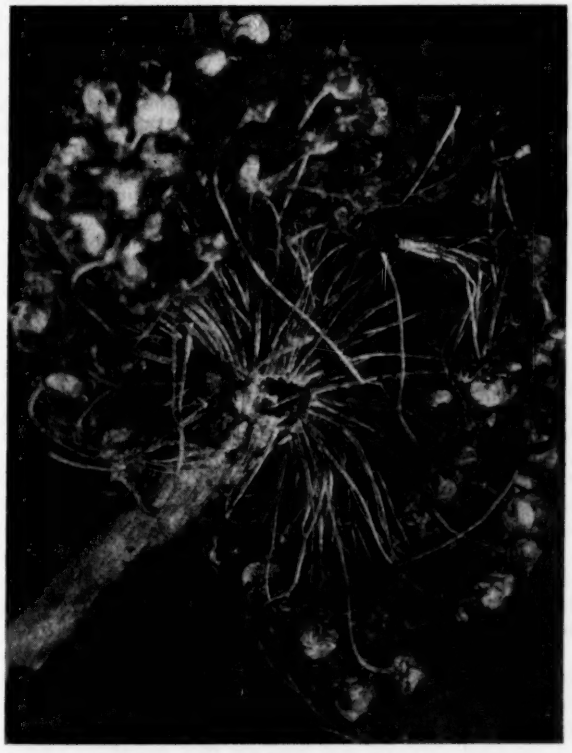

FIG. 2.—Seed head with piece of stem broken away to show puparium.

parasite when placed in the rearing cabinet after refrigeration for 90 days. The parasite was identified as *Aphaereta* sp. near *auripes* (Provancher) and the puparia as those of *Hylemya antiqua*.

Twenty-nine flies emerged from the puparia treated with DDT; the majority showed tremors and all died within 2 days after emergence. No flies emerged from the puparia treated with dieldrin. Sixty-seven flies emerged from the untreated puparia.

Puparia can be removed from the seed by washing as a final cleaning process. The fact that washing is practised in many seed-growing areas may explain why there are few if any references in the literature to puparia in the seed heads.

SUMMARY.—Six species of Diptera were obtained from the seed heads and stems of onions and from commercial seed. There was no association between the length of the stem cut with the seed head and the number of maggots or puparia; threshing reduced the numbers markedly. Nearly twice as many flies emerged from puparia not subjected to threshing as from those subjected to threshing, whether the insects underwent diapause or not. A braconid parasite, *Aphaereta* sp. near *auripes* (Provancher), emerged from undersized puparia of the onion maggot, *Hylemya antiqua* (Meig.). Treating puparia mixed with seed with dieldrin or DDT prevented flies from emerging or killed them on emerging.

REFERENCE CITED

Finlayson, D. G., and R. H. Handford. 1954. Experiments on control of the onion maggot, *Hylemya antiqua* (Mg.), in the interior of British Columbia. Canad. Jour. Agr. Sci. **34**(4): 385–8.

BOOK REVIEWS

INSECT PESTS OF FARM, GARDEN AND ORCHARD, 5th Edition. By Leonard Marion Peairs and Ralph Howard Davidson. John Wiley & Sons, Inc., New York City. Chapman & Hall, Ltd., London. 1956. 6×9¼ inches. viii+661 pp. 577 figs. $8.50

It is not completely fair to consider this book to be merely a new edition because as indicated by the author, Dr. Ralph H. Davidson, in the preface: "Most of the text has been re-written." The reviewer has verified this statement by a comparison with the fourth edition. The first 20 pages are entirely new and constitute a proper introduction, or review for students having had an introductory course, for what follows. There is a reduction of 70 in the number of illustrations from the previous edition but the book is amply illustrated largely with the old cuts. There are no illustrations of horse-drawn spraying equipment in this edition.

The chapter divisions beginning with Chap. 8 (p. 121) follow the general pattern of the divisions in the previous editions from Chap. 6 (p. 87) to the end. There was no need to change that pattern for time and use have proved it to be quite satisfactory. Of course, the most pronounced change in this edition is the incorporation of the new insecticides in the controls. The chapter on Insecticide Formulations, Applicators and Dilution Tables, though brief, will be useful.

It is obvious that the up-to-date author made a Herculean effort to add new facts about the life histories, habits, and controls of the species listed. Though of course limited to brevity, the author appears to have done remarkably well. Obviously the author is a successful teacher in a course or courses making use of this text. He has therefore been gathering this information for years. The subject is changing and growing so fast that no such writing can be fully up-to-date when it appears in print. No doubt the spotted alfalfa aphid assumed major importance in southwest U. S. after the manuscript was sent to the publisher.

We are glad to see that the publisher did not relegate some of the species to smaller print as was done in the previous edition. A subject is not second in importance when one is seeking information specifically on it.

It is natural for each of us to examine the accounts of species best known to us. In my case, the author passes with a high mark. I wish, however, that future authors would correct the old-time explanation of fig. 30. The detailed, large scale drawing of the adult chrysopid and head are very probably *Chrysopa oculata* Fitch, but the two larvae are probably *Chrysopa rufilabris* Burm. They are absolutely not *C. oculata*, as has been indicated by writers generally since Marlatt published his pear psyllid paper in 1895.

The reviewer anticipates that this new edition will be as useful for students and for use by the general public as previous editions have been. They have been widely used as a text partly because the book, or sections of it, have not been too large, nor too detailed, for use in teaching; and because it has been a readable, well illustrated, readily understood book. This one maintains these same high qualities. Dr. Davidson and the publishers are to be congratulated for giving this formerly extensively used book in our field a new lease on life.

ROGER C. SMITH

REVISAO DO COMPLEXO CYRTORHINUS FIEBER-MECOMMA FIEBER (HEMIPTERA-HETEROPTERA, MIRIDAE), by José C. M. Carvalho and T. R. E. Southwood. Bol. Mus. Paraense Emilio Goeldi Tomo XI, Fasc. 1, pp. 72, 25 plates (172 figs.), 3 charts, 1955. Caixa Postal 399; Belém, Para; Brazil. ["Please exchange"]

This is a world revision of an economically important group of plant bugs. According to our present knowledge all species are predators on insect eggs inserted into plant tissues. One species has been introduced into the Hawaiian Islands for control of the taro planthopper and another is credited with saving the Island's sugar industry from the ravages of the sugar cane leafhopper. The paper provides biological control workers with the names, description and distribution of 30 potentially useful agents for the control of a variety of insects that insert their eggs into plant tissues.

In addition to describing one new genus, five new species and three new subspecies, the authors resolved a taxonomic problem that has disturbed students of mirid classification for many years. Past treatments of *Cyrtorhinus* placed the genus in the subfamily Orthotylinae although it was known that some species had bristle-like arolia between the claws rather than the flap-like arolia characteristic of Orthotylinae. Otherwise the species seemed to resemble each other closely. By studying the genital structures of both sexes the authors have shown that the resemblance is superficial and that those species with bristle-like arolia belong to the subfamily Phylinae where they take the generic name of *Tytthus*. As a result of the study the correct name of the predator on the sugar cane leafhopper is *Tytthus mundulus* (Breddin), while that of the predator on the taro planthopper remains *Cyrtorhinus fulvus* Knight.

R. I. SAILER

Laboratory Comparison of Eight Organic Phosphorus Insecticides as Larvicides against Nonresistant House Flies[1]

DONALD A. LINDQUIST[2] and R. W. FAY[3]

Resistance to many chlorinated hydrocarbon insecticides has focused attention upon the organic phosphorus compounds for house fly control. This paper discusses laboratory comparisons of various organic phosphorus compounds applied as emulsions to the surface of fly larval breeding media. Two factors were varied (a) the concentration of the test emulsions and (b) the age of the house fly culture at the time of treatment. The following eight organic phosphorus insecticides were studied: malathion, Bayer L 13/59, demeton, NPD, Bayer 21/199, parathion, EPN, and Diazinon.

PROCEDURE.—For each fly test culture, one kilogram of finished CSMA larval-rearing medium[4] was placed in a battery jar 6 inches in diameter and 9 inches high. The jar was covered with a muslin top to prevent supervenient oviposition. One day later 25 mg. of newly hatched house fly larvae were added to each culture. The susceptibility of larvae at various ages was checked by insecticide treatments of the cultures under one of the following four treatments: "−1" designates treatment of fresh culture media 1 day before larval introduction (newly hatched larvae); "1" designates treatment 1 day after larval introduction (medium size larvae, mostly second instar with a few third instar larvae); "3" designates treatment 3 days after larval introduction (large larvae, all third instar larvae); and "5" designates treatment at 5 days after larval introduction (mainly pupae, with few third instar larvae).

Insecticide emulsions were sprayed at 10 lbs. p.s.i. pressure using a conventional nasal atomizer. The treatment was applied to the surface only and was not stirred or mixed into the culture. Xylene concentrates of 0.5% insecticide and 2% *Triton X-155* were emulsified with water for applications of 0.5, 1.0, 2.5, 5.0, and 10 mg. of insecticide per sq. ft., similar concentrates of 1% insecticide were emulsified for applications of 20, 30, and 50 mg. of insecticide per sq. ft., and concentrates of 5% insecticide were emulsified for dosages of 100 and 200 mg. of insecticide per sq. ft. The emulsions were prepared so that an application of 5 ml. on the 0.2 sq. ft. area of each culture would give the desired dosage.

Cultures were held for 1 week to permit pupal development. Pupae were then recovered by removing the top portion of the mash, spreading it out to dry on toweling, and finally winnowing off the dry mash with an electric fan. The pupae were counted and held for adult emergence. Counts were made during the next 7 days of the normal adults and of the defective or incompletely emerged adults.

The evaluations given are based on four cultures, two replicates on each of 2 days. In each group of 10 cultures, two were designated as checks and were sprayed with a xylene-*Triton X-155* emulsion equivalent in solvent concentration to the highest dosage for the test group. Check cultures averaged 300 pupae and when checks produced poorly the entire 10 cultures were discarded. Approximately 750 cultures were used in the evaluations given in the following tables.

Effectiveness of the insecticides was based on three values: (A) the pupae produced, (B) the total adult flies produced, and (C) the number of normal viable adults produced. Based on these values the per cent reduction as larvae, as pupae, and as defective adults were obtained by the following formulae:

$$\text{Per cent reduction as larvae} = \frac{(\text{Check pupae} - \text{test pupae})(100)}{\text{Check pupae}} = A$$

$$\text{Per cent reduction as pupae} = \frac{(\text{Total check adults} - \text{total test adults})(100)}{\text{Total check adults}} - A = B$$

$$\text{Per cent reduction as adults} = \frac{(\text{Check normal adults} - \text{test normal adults})(100)}{\text{Check normal adults}} - A - B = C$$

$$\text{Total per cent reduction} = A + B + C$$

RESULTS.—The total per cent reduction obtained with the treatment of fly cultures of each test age by known dosage of the test insecticides is summarized in table 1. The insecticides, in the order of their increasing effectiveness, are arranged from left to right in the table. Generally a given dosage of insecticide was most effective when applied to "1" cultures, next best on "−1" cultures, and less effective on "3" cultures. None of the insecticides showed any marked effectiveness on "5" cultures even at dosages of 200 mg. insecticide per sq. ft.

Malathion, Bayer L 13/59, and demeton were quite similar in effectiveness, requiring applications of 10 and 20 mg. per sq. ft. for more than 90% mortalities on "−1" and "1" cultures and failing to give 90% mortalities on "3" cultures even at 200 mg. insecticide per sq. ft. Complete mortalities in "−1" cultures were obtained with applications of 100 or 200 mg. per sq. ft. and in "1" cultures with 20 to 50 mg. per sq. ft. with these three insecticides.

NPD and Bayer 21/199 were slightly superior to the group above since more than 90% mortality was obtained on "3" cultures with applications of 100 and 200 mg. of insecticide per sq. ft. Both chemicals produced better than 90% mortality in "−1" cultures when applied at

[1] From the Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, Atlanta, Georgia. Accepted for publication January 6, 1956.

[2] Assistant Sanitarian (R), now Graduate Assistant, Department of Zoology and Entomology, Iowa State College, Ames, Iowa.

[3] Entomologist, Technical Development Laboratories (CDC) Savannah, Georgia.

[4] Chemical Specialties Manufacturers Association Standard Fly Rearing Medium prepared by the Ralston Purina Company, St. Louis, Mo. Nondiastatic malt extract, yeast suspension, and water were added to the dry mash for finished medium. The use of trade names is for the purpose of identification only and does not represent endorsement by the U. S. Public Health Service.

Table 1.—Total per cent reduction as larvae, purpae, and defective adults obtained by surface applications of different organic phosphorus compounds on various house fly cultures.

Age of[a] Culture	Dosage (Mg./Sq. Ft.)	Total Per Cent Reduction from Treatment with							
		Malathion	Bayer L 13/59	Demeton	NPD	Bayer 21/199	Parathion	EPN	Diazinon
−1	0.5								5
	1.0				10	14	6		70
	2.5				58	52	69	9	94
	5.0	53	36	49	78	77	90	92	98
	10.0	63	69	98	93	91	94	96	98
	20.0	95	92	93	95	99	97	96	100
	30.0	97	96	97	90	97	96	97	
	50.0	92	98	99	100	100	97	97	
	100.0	97	100				99	100	
	200.0	100					100		
1	0.5								28
	1.0		47		24	26	28	13	96
	2.5		63	49	97	59	47	62	96
	5.0	61	91	65	100	90	91	93	100
	10.0	98	88	94		99	91	98	
	20.0	100	93	98		99	98	100	
	30.0		98	100		100	100		
	50.0		100						
3	2.5						16	25	6
	5.0				0		57	75	97
	10.0			12	23	6	92	83	94
	20.0		24	36	26	65	94	94	91
	30.0		55	58	51	80	95	94	97
	50.0	33	66	84	87	96	97	94	100
	100.0	52	56	84	88	100	99	96	
	200.0	76	71	77	95		100	96	
5	50.0								13
	100.0								10
	200.0	1	10	6	3	16	4	3	20

[a] Age of culture refers to age of larvae at time of treatment. −1 is treatment 1 day before introducing newly hatched fly maggots, 1, 3 and 5 refer to age of larvae in days.

10 mg. of insecticide per sq. ft., and complete mortality at 50 mg. of insecticide per sq. ft.

Parathion and EPN showed better larvicidal action than the previous compounds since dosages of either chemical at 5 mg. per sq. ft. gave 90% or higher mortality in "−1" cultures. For complete mortality, however, the dosages of 200 mg. of parathion, or 100 mg. of EPN, per sq. ft. required were no less than those of the previous chemicals tested. Against "1" cultures parathion and EPN were essentially equal to NPD and Bayer 21/199. Against "3" cultures only 10 and 20 mg. per sq. ft. of parathion and EPN respectively were required for more than 90% control.

The best compound of the group tested was Diazinon which was very active against the "−1" "1," and "3" cultures. More than 90% mortality was obtained in "−1", "1" and "3" cultures with 2.5, 1, and 5 mg. Diazinon per sq. ft. respectively. Complete kill was obtained with 20 mg. of Diazinon per sq. ft. in "−1" cultures, with 5 mg. per sq. ft. in "1" cultures, and with 50 mg. per sq. ft. in "3" cultures.

The total per cent reduction with Bayer 21/199 larvicide applications broken down into its components of reduction as larvae, as pupae, and as defective adults, is typical of the organic phosphorus compounds tested (Table 2). With treatment of "−1" and "1" cultures, essentially all mortality occurred as larval reduction with practically no delayed effects expressed in pupal mortality or in defective adults. With treatment of "3" cultures, however, a considerable portion of the mortality occurred after the insects reached the pupal stage, and a minor additional effect was expressed as defective adults. Treatment of "5" cultures evidently affected only the larvae in the mixed larval and pupal cultures.

Discussion.—In the present evaluations, emphasis has been given to the action of the organic phosphorus compounds applied to larval cultures. Any residual effectiveness against emerging adults was eliminated by the mechanical separation of the pupae from the treated culture material. In field applications it is possible that even higher effectiveness may be shown, especially by compounds having appreciable residual activity.

In similar work with a series of halogenated hydrocarbon insecticides (McCauley *et al.* 1955) BHC, aldrin, and chlordane showed high activity against the larvae with some delayed activity expressed in pupal mortality and defective adults. These results were quite comparable to those with the organic phosphorus compounds tested. DDT, dieldrin, and toxaphene, however, allowed considerable numbers of larvae to pupate before succumbing to the toxic effects of the insecticides, and higher proportions of defective adults were evident also.

Table 2.—Per cent reduction as larvae, as pupae, and as defective adults from surface applications of Bayer 21/199 made on various house fly cultures.

Age of Culture[a]	Dosage (Mg./Sq. Ft.)	Per Cent Reduction		
		As Larvae	As Pupae	As Defective Adults
−1	1.0	13	1	0
	2.5	50	2	0
	5.0	73	4	0
	10.0	89	2	0
	20.0	98	1	0
	30.0	96	1	0
	50.0	100	—	—
1	1.0	23	2	1
	2.5	56	3	0
	5.0	88	2	0
	10.0	98	1	0
	20.0	99	0	0
	30.0	100	—	—
3	10.0	0	5	1
	20.0	19	43	3
	30.0	20	60	0
	50.0	44	50	2
	100.0	69	31	—
5	200.0	15	1	0

[a] Age of culture refers to age of larvae at time of treatment. −1 is treatment 1 day before introducing newly hatched fly maggots, 1, 3 and 5 refer to age of larvae in days.

Based on considerations of both insecticidal activity and toxicity hazards, Diazinon appears to be the larvicide of choice in the group tested. It should be emphasized that dosages of 5 or 10 mg. of Diazinon per sq. ft. gave high mortalities under laboratory conditions regardless of the stage of larval development in the culture. With the next most efficient larvicides EPN and parathion, the greater toxicity hazard makes the application levels of greater importance. Here, while dosages of 5 mg. per sq. ft. of either EPN or parathion gave high mortalities of small or medium size larvae in "−1" or "1" cultures, the results on large larvae in the "3" cultures were mediocre, and dosages of at least 10 mg. of insecticide per sq. ft. would be required. In these cases it might be advantageous to employ periodic applications of the two insecticides at the level of 5 mg. per sq. ft. and endeavor to cover new infestations rather than to employ an over-all application at the higher dosage. With the other compounds tested the difference in application levels necessary for high mortalities between the young "−1" and "1" cultures and the mature "3" cultures is even more marked, and repeated low dosage application covering infestations appears worth while rather than over-all treatments of all breeding media. With repeated low dosage applications the effects of larvicidal treatments on the adult fly population would be somewhat delayed in appearance.

ACKNOWLEDGMENT.—The assistance of G. C. Morris, III, in conducting the laboratory phases of these experiments, is gratefully acknowledged.

SUMMARY.—Laboratory comparisons of the larvicidal efficiency of eight organic phosphorus insecticides were made using surface applications of various xylene emulsions on breeding material containing various size fly larvae. Diazinon proved to be the most effective, giving better than 90% mortalities of small, medium, and large house fly larvae at application rates of 5 mg. of Diazinon per sq. ft. In general the compounds were more effective on the small or medium size larvae than on the large larvae and were relatively ineffective against fly pupae. In order of decreasing effectiveness the compounds were Diazinon, EPN, parathion, Bayer 21/199, NPD, demeton, Bayer L 13/59, and malathion.

REFERENCE CITED

McCauley, Robert M. Jr., Mary M. Grainger, Donald A. Lindquist, and Richard W. Fay. 1955. Laboratory comparison of some insecticides as larvicides against nonresistant house flies. Jour. Econ. Ent. **48**(3): 269–73.

The Effect of Biting Fly Control on Milk Production[1,2]

PHILIP GRANETT and ELTON J. HANSENS, *Rutgers University, New Brunswick, N. J.*

Although some degree of protection can be provided dairy cattle from the attack of biting flies, relatively little definite information is available about the effects of such protection on milk production. Decker (1955) has recently summarized the pertinent literature dealing with this phase of the economics of fly control and concludes that the adverse effects of biting flies may be offset by adequate treatment of the animals. He points out that, although other factors may also be involved, a steady rise in milk production during the past 25 years has been accompanied by an increased use of fly spray on dairy cattle. The difficulties involved in accurately determining the effectiveness of protective treatments are numerous, involving both management and fly population. This paper reports results of a limited investigation which was undertaken to determine the actual effect of biting fly control on milk production under New Jersey conditions.

PROCEDURES AND MATERIALS.—Two typical farm herds were selected in Salem County, where a large population of biting flies and mosquitoes would normally be expected during the summer season. The cows were num-

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, the State University of New Jersey, Department of Entomology, New Brunswick. Accepted for publication January 6, 1956.

[2] Investigations conducted under a research grant sponsored by Carbide and Carbon Chemicals Company at Rutgers University.

bered and, in early June, daily counts were begun of biting flies annoying the animals in the pasture. At this time horn flies, *Siphona irritans* (L.), were found most abundant. Later in June and July horse flies, *Tabanus* spp. and deer flies, *Chrysops* spp., were also present in moderate numbers. Stable flies, *Stomoxys calcitrans* (L.), became abundant in July and August. Mosquitoes, mostly of salt marsh species, were also abundant in August. Fly counts were made once each day, Monday through Friday.

In addition to observing pest conditions in the field, morning and afternoon milk records throughout the week were kept for each cow. As a result of the preliminary observations, the herds were divided into three groups of approximately equal milk production and susceptibility to fly attack. On one farm, designated as Farm 1, each of the three groups consisted of five Holstein cows, and on the other farm, Farm 2, each group contained four Guernsey cows. Two of these groups on each farm were sprayed by means of a 5-gallon knapsack sprayer with water emulsion formulations; the third group was left untreated as a check.

The treatments were as follows:

Group A—Formulation containing 10% methoxychlor, 8% Triton X-188, and 82% Sovacide 544-C, diluted 1 to 19 parts water, approximately 1 quart applied per animal.

Group B—Formulation containing 5% methoxychlor, 50% butoxy polypropylene glycol (Crag Fly Repellent), 8% Triton X-188, and 37% Sovacide 544-C, diluted 1 to 9 parts water, approximately 1 quart applied per animal.

Group C—Untreated.

On the day the cows were sprayed, all the Group C (untreated) cows were released from the barn after they were milked. The 10% methoxychlor formulation was diluted and applied to all the Group A cows. These were then released from the barn. Then the methoxychlor-butoxy polypropylene glycol formulation was diluted and applied to the Group B cows. The cows were then free to go the the pasture. The first application was made on June 14, in the morning. The second application, a week later, was also made in the morning. On subsequent applications, cows on Farm 1 were sprayed in the morning after milking and on Farm 2 in the late afternoon or early evening. Treatments[3] continued at weekly intervals until the last week in August.

RESULTS AND DISCUSSION.—Direct comparison of the three groups for milk production on Farm 1 was limited to 5 weeks because one of the cows in the untreated group came to the end of its lactation period after the fifth weekly treatment. This occurred with other cows in the other groups in the following weeks. On Farm 2, several cows came to the end of their lactation period after the seventh treatment and comparison of the groups on this farm was limited to 7 weeks. A summary of the average number of flies per cow per day for each day following weekly spraying during the test period is shown in table 1. A summary of the average milk production per cow per day for each day following spraying during the comparison period is shown in table 2.

The results indicate that for most of the period the sprayed groups on both farms were superior to the unsprayed group. The formulation containing methoxychlor and butoxy polypropylene glycol appeared to be somewhat better than the formulation containing methoxychlor alone. To be able to form a more definite opinion on the significance of the trends noted, the figures were submitted to an analysis of variance test.

Table 1.—Daily average number of flies[a] counted on treated and untreated groups of cows during a 7-week period.

Days After Spray	Farm 1 Average No. of Flies per Cow per Day on Group[b] A	B	C	Farm 1 Difference Significant Between Groups	Farm 2 Average No. of Flies per Cow per Day on Group[b] A	B	C	Farm 2 Difference Significant Between Groups
1st	8.1	5.1	20.1	A & C B & C	11.8	7.0	19.1	A & C B & C
2nd	6.2	3.1	15.2	A & C B & C	14.2	12.1	26.3	None
3rd	9.9	6.5	17.1	None	14.1	14.4	20.1	None
6th	18.3	11.4	22.1	None	20.2	17.5	28.8	None

[a] Primarily horn flies and stable flies.

[b] Group A—10% methoxychlor formulation diluted 1–19 parts water.
Group B—F_{51} (5% methoxychlor, 50% butoxy polypropylene glycol) formulation diluted 1–9 parts water.
Group C—Untreated.

Table 2.—Daily average milk production from treated[a] and untreated groups of cows.

Days After Spray	Farm 1 Daily Average Pounds of Milk per Cow for 5-week period A	B	C	Farm 1 Difference Significant Between Groups	Farm 2 Daily Average Pounds of Milk per Cow for 7-week period A	B	C	Farm 2 Difference Significant Between Groups
1st	25.9	27.2	25.8	None	17.3	17.8	18.0	None
2nd	27.6	29.4	25.2	A & C B & C	18.0	19.6	18.3	None
3rd	28.8	29.8	26.5	B & C	18.3	19.0	17.7	None
4th	27.8	29.1	26.1	None	18.2	19.5	19.1	None
5th	27.8	28.9	26.1	None	17.6	18.2	18.2	None
6th	26.8	28.8	26.1	None	17.4	18.6	18.3	None

[a] See table 1 for treatments.

Fly control.—On Farm 1, there was significant difference in number of flies on the cows between the treated and untreated groups but not between the two treatments on the first 2 days following spraying. On the 3rd and 6th days, fly control on the methoxychlor plus butoxy polypropylene glycol tested group just missed being significantly better than the other treated group.

On Farm 2, there was significant difference only on the first day in numbers of flies on the treated cows as compared to the untreated cows. There was no significant difference between the two treatments.

The apparently less effective fly control at Farm 2 may have been due to various causes such as location, farm management, or cow susceptibility. In addition, for 5 of the 7 weeks under consideration, the cows at this farm were sprayed in the evening whereas at Farm 1 the cows were sprayed the next morning. Counts of flies were generally made each afternoon and consequently the counts

[3] Acknowledgement is made of the dependable work of Mr. Ed. Gant for applying the sprays and making the fly counts.

would tend to be lower on Farm 1 because of the shorter exposure period. This effectiveness continued on Farm 1 for the methoxychlor plus butoxy polypropylene glycol for the second day.

Milk production.—Although no significant difference was found between the groups on Farm 1 on the first day, a definite difference was observed on the second day between the treated and untreated groups but not between the treatments. On the third day, there was a significant difference between the group treated with methoxychlor plus butoxy polypropylene glycol and the untreated group. Group A, sprayed with methoxychlor alone, apparently was not affected sufficiently by fly control to produce more milk. No significant difference was observable on succeeding days.

For the 2-day period in which significant difference was shown by the methoxychlor plus butoxy polypropylene glycol group on this farm, there was an average increase per cow of 7.5 pounds of milk or for the 5-week period an average increase of 37.5 pounds. If the farmer gets 5 cents per pound for his milk the increase over the 5-week period would amount to $1.87. Cost of this spray formulation for this period of 5 weeks is estimated at 80 cents per cow. If only the 2-day period in which significance was shown is taken into account, use of the methoxychlor plus butoxy polypropylene glycol formulation would result in about $1.00 increase in revenue for the farmer because of increased milk production. The cost of the methoxychlor formulation without the butoxy polypropylene glycol is estimated at 20 cents per cow over the 5-week period, but since significant difference was shown for only 1 day, the increase in profit over untreated animal was 40 cents per animal. In any event, it is apparent that spraying is worth while.

It should be pointed out that even the untreated animals benefited from the spray treatments and consequently milk production on these animals was not as adversely affected by pest conditions as would be evident on an unsprayed herd. It is also possible that had the cows been sprayed more often, additional increases in milk production might have resulted.

On Farm 2 although the methoxychlor plus butoxy polypropylene glycol treatment was apparently consistently better in milk production, there was no significant difference between the check and this treatment. It is probable that aside from farm management and insect control practices, more than four cows per group is needed to show significant difference in milk production. It is hoped that further work of this sort on a larger scale will be conducted in the near future.

SUMMARY AND CONCLUSIONS.—Each of two herds located in Salem County, N. J. were divided into three groups almost equally susceptible to biting fly attack and providing similar milk production. Two of the groups in each herd were sprayed at weekly intervals with two water emulsion formulations. One of the formulations contained methoxychlor as the active ingredient and the other contained methoxychlor plus butoxy polypropylene glycol (Crag Fly Repellent). The third group was left as an untreated check. Daily observations were made of the number of biting flies on the individual cows and the milk produced. For a period of 5 to 7 weeks, it was found that a significant drop in fly numbers (horn, stable, and horse flies) was observable on the treated groups 1 to 2 days after spray applications. An increase in milk production was found on one farm for 1 day following the use of the methoxychlor formulation and for 2 days following the use of methoxychlor plus butoxy polypropylene glycol. It was estimated that the increase in milk production more than paid for the cost of fly protection.

REFERENCES CITED

Decker, George C. 1955. Livestock Fly Control—Does it Pay? Soap. and Chem. Specialties Part 1, Vol. **XXI**, No. 6, pp. 142, 143, 145, 147. Part 2, Vol. **XXI**, No. 7, pp. 151, 153. 155.

Codling Moth Control on Pears 1954–55[1]

HAROLD F. MADSEN, *University of California, Berkeley*

Since the advent of DDT for control of the codling moth, *Carcocapsa pomonella* (L.), the spray program on Bartlett pears in California has consisted of a petal fall application followed by a second spray 21 to 30 days later. On late varieties, such as Comice, Hardy and Winter Nellis, a third spray is applied around July 1. Following the report of codling moth resistance to DDT by Cutright (1954), studies were begun on possible substitute materials for codling moth control. Since that time Glass & Fiori (1955) have also reported resistance in New York State.

Several materials have been tested by other workers as substitutes for DDT in the codling moth program. Glass (1954), and Dean & Newcomer (1954) have reported favorable results with the use of methoxychlor. Several workers, including Hamilton *et al.* (1953) Patterson & MacLellan (1954) and Clancy (1955) have shown that ryania not only gave good control of codling moth, but in addition, did not destroy predators of spider mites and other fruit pests.

1954 STUDIES.—In 1954, a plot was established in an isolated 15-acre block of Bartlett pear trees near Penryn, California. Each plot was one acre in extent, replicated three times, and applications were made with blower sprayer equipment. At each application, applied gallonage averaged 500 gallons per acre. Four seasonal sprays were applied—April 19, April 26, May 17, and June 29.

At harvest, counts were made on the number of fruit injured by codling moth by examining 2,000 fruit per treatment in each of two pickings. In addition to codling

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moth, grape mealybug, *Pseudococcus maritimus* (Ehrh.) and a lepidopterous leaf miner, *Lithocolletis sp.* were also present. In the case of the mealybug, plots were evaluated by recording the number of pears with mealybug present in the calyx end. With the leaf miner, a final evaluation was made on September 15 by counting the number of mines showing emergence of adults on 200 leaves per plot. As the pupal cases remain attached to the remnants of the old mines, this count shows the number of leaf miner larvae which have reached maturity and is a measure of the control obtained.

Materials used, dosages, and the harvest counts are summarized in table 1. The results showed that Diazinon, DDT, methoxychlor and ryania all gave comparable control of codling moth, having less than 1% wormy fruit at harvest. Lead arsenate, although providing commercial control, was not as effective as the other materials mentioned.

Diazinon provided excellent control of mealybug, all fruit examined being free of infested calyx ends. The lead arsenate and ryania plots showed less mealybug infested fruit than DDT and methoxychlor, but the reduction was not marked.

Ryania provided complete control of the leaf miner, as not a single larva reached maturity on this plot. Diazinon also reduced the leaf miner to a low level as compared with DDT, methoxychlor and lead arsenate. None of these latter materials prevented leaf miner development. One of the reasons for using ryania in this test was to see if the material would be less destructive to the parasites of leaf miner than is the case when chlorinated hydrocarbon insecticides are used. Ryania, however, so completely controlled the miner that no larvae reached the stage where parasitism normally takes place.

The European red mite, *Metatetranychus ulmi* (Koch), and the two-spotted spider mite, *Tetranychus telarius* (L.), were also present in this orchard, and it was necessary to add an acaricide to the June 29 spray in all plots with the exception of Diazinon. The use of this latter material kept the mite populations at a low level for the season. There were less mites present on the plots sprayed with ryania and lead arsenate than was the case in the DDT and methoxychlor plots. Observations, however, did not indicate the difference to be due to predators, as a slight degree of control was obtained following applications of ryania and lead arsenate. The reduction in mite population was not enough, however, to eliminate the need of an acaricide in order to prevent foliage damage.

1955 STUDIES.—In 1955, codling moth plots were established in three locations in California. One was at Courtland, one at Penryn, and the third at San Jose.

Courtland Plot.—At the end of the 1954 season a grower in the Courtland area reported that at harvest a large percentage of his fruit was heavily infested with codling moth. One area within the orchard had 60% wormy fruit. The orchard had received two applications of DDT according to the spray schedule, and a third application with the hormone a week prior to harvest. An investigation of the orchard showed the pattern of severe infestation to be a football-shaped area, 4 to 5 acres in extent, along one side of the orchard. In this area, overwintering codling moth were very numerous beneath the bark scales. In order to determine if resistance was the factor in this orchard, plots were established using DDT, ryania and Diazinon. Each plot was ½ acre in extent, replicated twice, and randomized within the area of heavy infestation.

In order to check on the codling moth flight pattern in this area, bait pans were used, and were set out in the plots to be treated with ryania. The pans were checked at weekly intervals, starting in April and continuing through August. An unusual pattern of flight developed in this orchard, as the first flight did not occur until the week of May 15. The flight continued through June and July, reaching peaks in early June and in early July. The older bait pan records for this area show a flight starting in April with peaks in late April and early May. The suggested timing for codling moth spray applications would have put the DDT sprays in April and May, leaving the month of June unprotected. It seemed from the bait pan records that the difficulty in codling moth control in this orchard might be due to timing rather than DDT resistance.

The experimental plots were treated on May 9, June 13 and July 7. According to the bait pan catches, the May 9 spray was too early, but at the time it was not realized that the codling moth flights were to be late. Materials were applied with blower sprayer equipment, and applied gallonage averaged 750 gallons per acre. At the time of the second application, many codling moth adults were observed in flight after the sprayer had passed.

The fruit was harvested in two pickings, the first on July 25, and the second on August 9. At each picking, fruit was examined for codling moth entries and stings. The materials used, dosages, and the harvest counts on 3,000 fruits per treatment are summarized in table 2.

DDT gave excellent control in this plot, having only 0.5% wormy fruit at harvest. In this same area, 60% wormy fruit was encountered the previous season. It seems evident therefore, that the difficulty in control was due to the improper timing of spray applications, and that resistance was not a factor. Ryania also gave good control of the codling moth, but Diazinon was only fair, having better than 3% wormy fruit at harvest.

The flight pattern of codling moth in this orchard raises some interesting possibilities. The late flight may have been due to a very cool spring which was general throughout California in the 1955 season. However, evidence indicates that the same flight pattern must have been present during the 1954 season, otherwise the grower's DDT program should have given control. It is

Table 1.—Summary of Harvest Fruit Counts[a] of Bartlett Pears. Penryn, 1954.

Material	Dosage per Acre (Pounds)	Per Cent Codling Moth		Per Cent Mealybug Infested	Leaf Miner[b]
		Worms	Stings		
Lead arsenate	30	2.1	1.6	9.0	176
Ryanicide 100	30	0.1	0.7	9.0	0
DDT, 50%	10	0.2	0.7	13.0	220
Methoxychlor, 50%	10	0.4	0.6	16.0	224
Diazinon, 25%	15	0.2	0.5	0.0	36

[a] Counts made July 14 and July 28.
[b] Emerged mines per 200 leaves on September 13.

Table 2.—Summary of Codling Moth Plots at Courtland, California, 1955.

Wettable Powder	Pounds per Acre	Harvest Fruit Counts[a]			
				Per Cent	
		Worms	Stings	Worms	Stings
Diazinon, 25%	14.2	106	44	3.5	1.4
Ryania, 100%	42.6	52	28	1.7	0.9
DDT, 50%	14.2	16	24	0.5	0.8

[a] Total from two pickings, July 25 and August 9.

possible that a late brood of codling moth has developed following an early season program of DDT applications, but flights will have to be checked over a period of several seasons before any conclusions can be drawn.

Two-spotted spider mite was the only other major fruit pest encountered in this plot. It was necessary to add an acaricide to the last spray on July 7 in both the DDT and ryania plots, and there was little difference in mite populations between the two treatments. The Diazinon-sprayed plot, however, did not develop a two-spotted spider mite population high enough to require the addition of a specific acaricide.

Penryn Plot.—A Bartlett pear grower located near Penryn, California had difficulty in controlling codling moth the preceding two seasons by following a DDT schedule. In order to check on possible resistance, acre-plots were established in this orchard, using DDT, ryania, Diazinon. The materials were applied by the grower with blower sprayer equipment, and the applied gallonage averaged 500 gallons per acre.

Bait pans were not set out in this orchard, therefore no records of codling moth activity were obtained. The first spray was applied on April 6, and the second on May 2, which is the normal timing for codling moth control in that area. Since the bait pan records at Courtland indicated a late flight, it was decided to apply a third spray on June 8 and a fourth on July 12 in case the same situation was present in this orchard. The fruit was harvested on August 5, and at that time, counts were made of codling moth entries and stings. The materials used, dosages, and the harvest fruit counts are summarized in table 3.

Both DDT and ryania gave excellent control in this plot, having less than 0.5% wormy fruit at harvest. The Diazinon plot, however, showed 6% wormy fruit which would have made field sorting necessary. The control obtained with DDT was considerably better than had been obtained for the past two seasons in this orchard. Here again, a late flight of moths may be the cause of the difficulty in control. The grower's normal program would not have included a June application, and apparently the June and July treatments were the key sprays in obtaining control.

Table 3.—Summary of Codling Moth Plots at Penryn, California, 1955.

Wettable Powder	Pounds per Acre	Harvest Fruit Counts		
		Fruit Examined	Per Cent	
			Worms	Stings
DDT, 50%	9.2	1415	0.3	0.2
Ryania, 100%	22.2	1583	0.2	0.3
Diazinon, 25%	11.5	1425	6.0	1.3

Two-spotted spider mite and leaf miner were also present in this orchard, and observations were made on the effects of the materials on these two pests. In the case of the two-spotted spider mite, it was necessary to add an acaricide to the DDT plot in both the June 8 and July 12 sprays. The two-spotted spider mite did not build up as rapidly on the ryania treated trees, but by July 12, it was necessary to use an acaricide in order to prevent foliage damage. The Diazinon plot did not develop injurious mite populations and did not require a treatment with a specific acaricide.

With the leaf miner, results were similar to those obtained in the 1954 season. Ryania gave excellent control of the leaf miner, and Diazinon also reduced damage to a low level. DDT did not show any effect on the miners and high populations developed by September.

San Jose Plots.—An orchard was located near San Jose, California, which had not received a codling moth spray for the past two seasons. The variety involved was Hardy pear, and an examination of the orchard in early spring disclosed large numbers of codling moth overwintering behind the bark scales. Since the owner of the orchard did not intend to harvest the fruit, it was decided to evaluate a number of compounds, dosages, and combinations which could not have been applied in a commercial orchard.

Bait pans were maintained in the orchard and sprays were timed according to the bait pan records. The first flights were recorded in early May, with a peak flight on May 15. The flights continued through June and early July.

Plots consisted of 12 trees each, replicated twice, which gave 24 trees per treatment. Materials were applied with conventional ground equipment and orchard guns, and applied gallonage averaged 560 gallons per acre. The first spray was applied on May 24, the second time on June 28 and a third time on July 18. The plots were located on one side of the orchard and the remainder of the area was untreated. This provided a heavy source of codling moth which could fly in from the untreated area.

The fruit was harvested on August 11, and since the orchard had received little or no care in previous seasons, the fruit crop was light. This further added to the chance for a heavy infestation. All the fruit of proper size and maturity were picked on August 11, and examined for worms and stings. The materials used, dosages and the harvest fruit counts are summarized in table 4.

DDT and ryania gave excellent control in this plot considering the degree of infestation present. Diazinon in both the wettable powder and emulsion concentrate formulations reduced the fruit damage to a considerable extent but did not provide a commercial degree of control. The combination of DDT and Diazinon showed better control than Diazinon alone, but it is not clear from this data if the degree of control obtained was due to the combination or whether the 5 lbs. per acre of DDT

Table 4.—Summary of Codling Moth Plots at San Jose, California, 1955.

Wettable Powder	Pounds per Acre	Harvest Fruit Counts		
		Fruit Examined	Per Cent	
			Worms	Stings
Diazinon, 25%	11.2	940	6.3	2.6
Diazinon, 25%[a]		643	4.8	1.9
Diazinon, 25% +DDT, 50%	5.6	750	2	1.6
Ryania, 100%	33.6	980	0.8	2.1
DDT, 50%	11.2	752	0.9	1.0
TDE, 50%	11.2	821	4.6	2.2
Stauffer 1303, 25%	11.2	731	2.3	0.9
Lead arsenate	22.4	703	14.3	2.1
Untreated Check		911	59.1	4.1

[a] Applied as emulsion concentrate at the rate of 11.2 pints per acre.

was in itself sufficient to reduce codling moth damage to this level.

TDE did not give as good control as DDT in this plot, and this is significant since in several California counties, TDE is frequently substituted for DDT in the codling moth program when orange tortrix is a problem. Lead arsenate did not give commercial control as it failed to prevent a high percentage of wormy fruit. Stauffer 1303[2] in this single test showed considerable promise in codling moth control and will be further tested.

SUMMARY.—In the 1954 season, codling moth plots established to find substitute materials for DDT, showed that Diazinon, ryania, and methoxychlor gave control comparable to DDT. Diazinon also controlled spider mites, leaf miner and mealybug, while ryania gave excellent control of leaf miner.

During 1954, reports were received which indicated two possible cases of DDT resistance. Investigations showed that resistance was not present and that improper timing of spray applications was responsible for the increased fruit damage. Tests in three orchards showed that DDT and ryania gave good control of codling moth, but Diazinon was not adequate under conditions where populations were high. Of the new materials, Stauffer 1303 showed considerable promise in codling moth control.

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REFERENCES CITED

Clancy, D. W. 1955. Effects of spray practices on apple pests and their natural enemies. Mountaineer Grower **25**(206): 11, 21.

Cutright, C. R. 1954. A codling moth population resistant to DDT. Jour. Econ. Ent. **47**(1): 189–90.

Dean, F. P., and E. J. Newcomer. 1954. Methoxychlor for codling moth control. Jour. Econ. Ent. **47**(5): 936–7.

Glass, E. H. 1954. Field evaluation of insecticides against codling moth. Jour. Econ. Ent. **47**(6): 1093–1101.

Glass, E. H., and Bart Fiori. 1955. Codling moth resistance to DDT in New York. Jour. Econ. Ent. **48**(5): 598–9.

Hamilton, D. W., S. A. Summerland, and E. J. Fahey. 1954. Codling moth control experiments, 1950–1953. Jour. Econ. Ent. **47**(5): 768–75.

Patterson, N. A., and C. R. MacLellan. 1954. Control of the codling moth and other orchard pests with Ryania. Ann. Rept. Ent. Soc. of Ontario **85**: 25–32.

[2] 0,0-diethyl S-p-chlorophenylthiomethyl phosphorodithioate.

Resistance to American Foulbrood in Honey Bees. I. Differential Survival of Larvae of Different Genetic Lines[1]

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For many years, American foulbrood (causative organism: *Bacillus larvae* White) was thought to be a disease from which no infected, honey-bee colony ever recovered. Occasionally, however, a beekeeper reported, on an observational basis, an apparent exception to the prevailing opinion. To investigate the question presented by these sporadic observations, Park, Pellett, and Paddock organized a carefully controlled experiment in 1935. One year later they reported (Park 1936) that some of the supposedly resistant colonies inoculated with the pathogen had eliminated all symptoms of American foulbrood and apparently recovered from an infection of *Bacillus larvae*. None of the check colonies recovered.

In 1936, a new generation of queens and drones was reared from 1935's most resistant colonies. An isolated apiary was established for natural mating of queens and drones. About one-third of the colonies comprising the new generation eliminated all symptoms of American foulbrood following inoculation (Park *et al.* 1937). The investigators concluded that resistance to American foulbrood exists in honey bees and is heritable.

The mechanisms of the resistance became an immediate problem and divergent opinions were expressed. The careful work of Woodrow (1941a, 1941b, and 1942), Woodrow & Holst (1942), and Woodrow & States (1943) provided

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[2] Our indebtedness to the late Dr. O. W. Park is gladly and gratefully acknowledged. He was in charge of bee research at Iowa State College from 1919 to 1923 and from 1925 until October, 1954. Dr. John W. Gowen, Department of Genetics, has helped us as a generous consultant on many occasions.

the first extensive evidence. It was concluded, "The data show that resistance to American foulbrood in the honeybee colony consists in its ability to detect and remove diseased brood before the causative organism, *B. larvae*, reaches the infectious spore stage in the diseased larvae." Tarr (1937) had already shown that the vegetative stage of the pathogen is not infectious. That being the case, housekeeping bees, conceivably, could remove and dispose of sick larvae safely if it were done promptly. Susceptible bees were observed to allow many dead larvae to remain in the comb long after spores had formed. Larvae from resistant and susceptible colonies were found by Woodrow (1942) to be equally susceptible to American foulbrood.

Observations and interpretations at the Iowa Station coupled with a perusal of pertinent literature indicated that such hygienic behavior alone was not a complete explanation of resistance. Consequently, a program was organized along new lines in 1951 to investigate the possible existence of other mechanisms of resistance. Certain guiding ideas not previously utilized in published bee-disease research were thought to be of prime importance for these investigations:

(1) Highly susceptible lines developed by selection through several generations are equally as useful as highly resistant ones similarly obtained, and both are essential if the mechanisms of resistance and their heredity are to be elucidated.

(2) Stocks of different origin are likely to have different potentialities of resistance or susceptibility.

(3) Selections following different types of inoculation are likely to result in the establishment of different mechanisms of resistance or susceptibility within the various lines. The three types of inoculation currently in use are feeding spores in sugar sirup to a colony, putting spores directly into the brood food of each larva, and replacing a piece of brood comb by a piece of infected comb. In each type, the chain of events conveying the pathogen to the host larva would show some differences and resistance mechanisms could intervene at different points.

The search for new mechanisms of resistance seems to have been appropriate. Sturtevant & Revell (1954) have recently announced the discovery of one such mechanism. Highly resistant colonies were found to remove a high percentage of the *Bacillus larvae* spores from infected sugar sirup fed to them. Less resistant colonies removed lower percentages. This mechanism would seem to be a very valuable one from a practical hygienic standpoint. Robbing of infected colonies might be less likely to lead to new cases of disease.

Working independently of Sturtevant and Revell, Thompson (1955) found in 1953 that nurse bees of a resistant strain from Hawaii were capable of preventing more of the larvae they nursed from succumbing to American foulbrood than were bees of the Van Scoy susceptible line. Whether this is a demonstration of reduced incidence of disease brought about by spore removal, or a demonstration of some other mechanism is not known. There is limited evidence that spore removal is involved.

The present report is concerned with a hitherto undiscovered mechanism of resistance. Three genetically separate lines of bees were found to show differential survival of larvae following inoculation of their larval food with spores of *Bacillus larvae*.

MATERIALS AND METHODS.—The line of bees designated as Van Scoy was started from a single, mated queen (a mated queen may be referred to also as a queen or a mating) obtained from Mr. Homer Van Scoy, Candor, New York, through Dr. William F. Coggshall of Cornell University, in 1950. The line has been somewhat inbred and selected for susceptibility to American foulbrood by the comb-insert method of inoculation (Park 1937, 1942). All matings in our line development and maintenance program have been by artificial insemination.

The Brown line was derived from the most resistant (by the comb-insert test) four of ten queens originally secured from Mr. Edward G. Brown, Sioux City, Iowa, in 1954. For many years, Mr. Brown has conducted a bold program in "natural" selection for resistance to American foulbrood. The colonies in one of his yards are allowed to rob the honey from infected combs which are brought to him for rendering. Over the course of many generations a high degree of resistance has been developed which Mr. Brown thinks is due to a physiological resistance in larvae. The Brown-line matings used herein consisted of queens and drones reared from the original four most highly resistant Brown queens.

The chartreuse line was derived from a single queen obtained from Mr. Harvey Walker, Urbana, Illinois, through Mr. Henry C. Dadant of Hamilton, Illinois, in 1949. This stock, which contains a mutation for chartreuse eyes (Rothenbuhler *et al.* 1953), has been somewhat inbred, with no planned selection until recently. Queens of the last generation were reared from larvae which were subjected to massive inoculations of their larval food with spores of *Bacillus larvae* when the larvae were less than 1 day of age. The survivors were expected to be those possessing higher larval resistance.

The two Van Scoy-line colonies produced by the two matings used in this experiment had been found to be highly susceptible when tested by the comb-insert method of inoculation. The two Brown-line colonies had been found to be highly resistant. The three chartreuse-line colonies had not been tested.

The first experiment was started during the latter part of June, 1955. Combs for the test were taken from storage and each selected queen was caged on a comb in in a comb cage made of perforated, queen-excluding, sheets of zinc. Queens were caged on June 24, between 3 and 6 o'clock P.M. About 24 hours later on June 25, between 3 and 5 o'clock P.M. the marked combs were removed from the cages and put into the second story (above a queen excluder) of a strong colony of commercial hybrid bees. By numbering the combs from left to right, the position in the second story that each test comb occupied was specified and is given in table 1.

On June 28, between late forenoon and 5 o'clock P.M. these combs were taken out of the rearing colony, given the experimental treatments, and returned to the rearing colony one at a time and in numerical order according to position. Two treatments were administered. The check treatment consisted in placing approximately $\frac{1}{4}$ cubic millimeter of tap water in the larval food of each larva by the use of a micro-syringe. In the case of a few larvae, larval food was not yet deposited in the cell. In such

instances the inoculation material was simply placed on the base of the cell. The syringe used was manufactured by the MacGregor Instrument Company on special order. An ordinary 25-gauge hypodermic needle was fitted to the end of the syringe. The syringe is actuated by a micrometer-screw head, one revolution of which delivers $\frac{1}{2}$ cubic millimeter. The spore treatment consisted in placing in the same way a like amount of tap water containing approximately 50,000 spores of *Bacillus larvae*. The spores in the form of a powder were generously supplied by Dr. A. P. Sturtevant of the U. S. Department of Agriculture's Bee Culture Laboratory at Laramie, Wyoming. Information as to the number of spores per unit of weight accompanied the spore powder, and from this information a suspension of spores in water could be made so as to contain about 50,000 spores per $\frac{1}{4}$ cubic millimeter. A single preparation of spores in water was used throughout each experiment.

Selection of larvae for each treatment was made as follows. A cardboard template was constructed so that five rows of about 30 cells each would be exposed in each of three areas. In most cases two of the areas on each side of the comb were found to contain brood and thus were suitable for experimentation. In most combs inoculated, only the three middle of the five available rows were considered because of better visibility in the three middle rows. By drawing one of three colored marbles from a box, it was decided whether row 2 would be given no treatment, check treatment (water), or spore treatment (water with spores). After row 2 was assigned, row 3 was assigned from the remaining two possibilities. Row 4 received the treatment left. In the case of the first two combs inoculated, assignment of treatments was to any of the five rows by a similar random process.

Two days after inoculation, June 30, all larvae were counted in the treated rows. Twelve days later, July 12, three or four days before expected emergence the cells in question were uncapped, if sealed, and inspected for healthy, dead, or missing individuals.

The second experiment was started on July 15, and was conducted in the same way with minor exceptions. In the second experiment, all test combs utilized had been stored overwinter and came from the same 1954 colony. Queens were caged on combs between 4 and 6 o'clock P.M. on July 15, and were removed between 8:30 and 10 o'clock A.M., July 16, for a shorter total period of egg laying than in Experiment I. On July 16, all combs were put into the same rearing colony used previously. Treatments were administered on July 19, between 10 A.M. and 5 P.M. An individual template was made of light-weight clear plastic for each comb in the second experiment. In all cases except one, only one side of a comb and only one large area per side was utilized. Rows were numbered from top to bottom. A coin was tossed to determine whether the first row would be given a check or spore treatment. Odd-numbered rows received one treatment even-numbered rows the alternate treatment. Counts of individuals were made at the same developmental periods as in the first experiment.

RESULTS AND ANALYSIS.—The results are presented in

Table 1.—Survival of honey-bee larvae, from various genetic lines, reared in the same colony.

LINE	MATING NUMBER	COMB POSITION	TREATMENT	BASE COUNT	SURVIVORS	PER CENT SURVIVORS	NON-SURVIVORS	OBSERVED AFB
			First Experiment					
Van Scoy	M 806	3	Check	44	44	100	0	0
	M 806	3	Spore	45	13	29	32	8
	M 808	9	Check	53	53	100	0	0
	M 808	9	Spore	48	8	17	40	21
Brown	M 899	7	Check	44	44	100	0	0
	M 899	7	Spore	77	55	71	22	13
	M 926	5	Check	85	84	99	1	0
	M 926	5	Spore	83	55	66	28	22
Chartreuse	M 869	2	Check	70	68	97	2	0
	M 869	2	Spore	76	36	47	40	22
	M 872	4	Check	52	49	94	3	0
	M 872	4	Spore	49	20	41	29	15
	M 876	6	Check	81	76	94	5	0
	M 876	6	Spore	73	31	42	42	27
			Second Experiment					
Van Scoy	M 806	7	Check	66	65	98	1	0
	M 806	7	Spore	108	31	29	77	45
	M 808	5	Check	80	74	92	6	0
	M 808	5	Spore	75	16	21	59	37
Brown	M 899	3	Check	65	62	95	3	2
	M 899	3	Spore	70	38	54	32	18
	M 926	9	Check	141	140	99	1	0
	M 926	9	Spore	144	103	72	41	23
Chartreuse	M 869	4	Check	57	54	95	3	0
	M 869	4	Spore	70	37	53	33	8
	M 872	2	Check	63	61	97	2	0
	M 872	2	Spore	54	26	48	28	9
	M 876	8	Check	79	76	96	3	0
	M 876	8	Spore	73	36	49	37	24

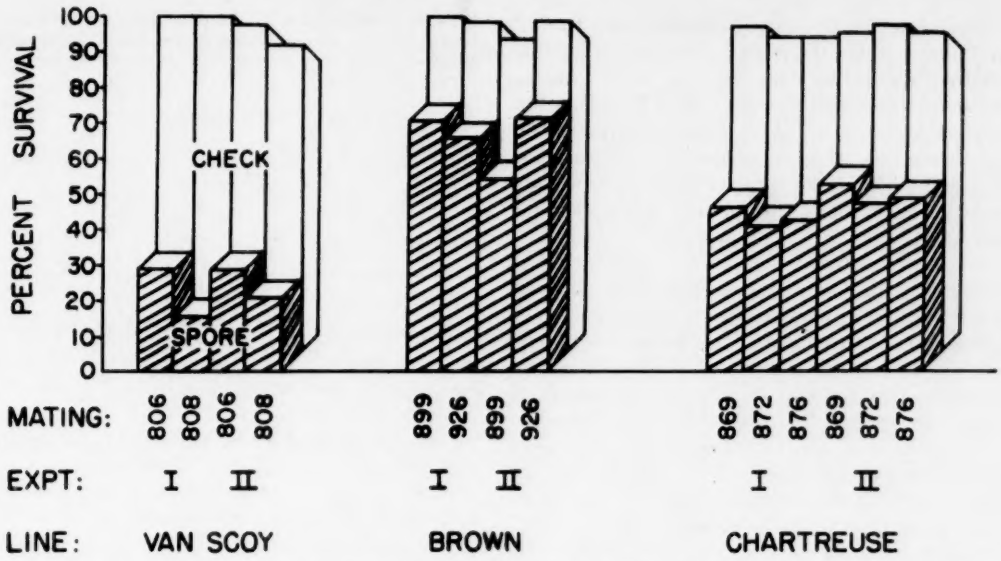

FIG. 1.—Per cent survival, in the same rearing colony, of check- and spore-treated larvae from three genetically distinct lines of bees.

figure 1 and table 1. Figure 1 is a graphic presentation of per cent survival in each treatment, in each comb (each mating number), in each experiment. Central tendency and variation in each line as well as differences between lines can be seen at a glance. The actual counts are presented in table 1. The base count represents the total number of larvae present within a treatment 2 days after the treatments were administered. At the final count, part or all of the larvae represented in the base count were found alive. The count of these is the figure in the survivor column. For easy comprehension of the results, the per cent of survivors has been calculated and is presented in the next column. The difference between the base and survivor counts constitutes the non-survivors and is so presented. Many non-survivors were simply missing from the cells as would be expected in view of the housecleaning proclivities of most bees. Nevertheless, some larvae dead of American foulbrood remained. These were recorded as observed AFB, although no account has been taken of observed AFB in the analyses presented herein because interest was primarily in survival and non-survival.

By way of general consideration of the data prior to more formal analysis, it may be pointed out that survivals in the check treatments were high in all lines. Especially, is this realized when comparisons with the spore treatments are made. Survivals in the spore treatments were lowest for the Van Scoy line, highest for the Brown line, and midway between the two for the chartreuse line. In the case of only one comb, M 899 in the second experiment, was any American foulbrood noted in a check. It is probable that the nurse bees spread the infection from the spore- to check-treated larvae. It is remarkable, but not unprecedented, that so little spread of the pathogen occurred (Woodrow 1942).

Results of first and second experiments are in agreement. —To investigate the question of whether the second experiment gave essentially the same results as the first, certain pooling of data within each experiment seems desirable. An inspection of the results of check treatments, for instance, indicates a close correspondence of the two experiments, but it is impossible to make a chi-square comparison of the experiments on the basis of each mating, or even each line, because of zero and low-numbered cells in the necessary 2×2 table. Considering only the check treatments, and keeping the two experiments separate, while pooling data from all matings and all lines, one obtains the totals 418, 11, 532, and 19 presented in table 2. A chi-square comparison of the first and second experiment can now be made for the check treatments after calculating expectations from border totals. Adjustment for non-continuity of the data was made. The chi-square value is 0.3713 which, with one degree of freedom, can be seen in a chi-square table to lead to a P value between 0.5 and 0.7. Greater deviations from expectations than those found in these results would be expected in a fraction of cases somewhere between 0.5 and 0.7 by chance alone. There is no evidence, consequently, that survival was different in the checks of the two experiments.

The spore treatments considered in the same way also result in a non-significant chi-square and they yield no evidence that survival was different in the spore treatments of the two experiments. In the case of the spore treatments it is possible to compare experiments on the basis of individual matings or lines. Some of these comparisons have been made and none was significant. On the basis of the demonstrated similarity in results, Experiments I and II are pooled for further analysis.

Table 2.—Comparisons of first and second experiments within treatments, pooling data from the three lines.

TREATMENTS	EXPERIMENTS	SURVIVORS	NON-SURVIVORS	CHI-SQUARE	P
Check	1	418	11		
	2	532	19	0.3713	.50–.70
Spore	1	218	233		
	2	287	307	0.0032	.95–.98

Matings within a line showed similar survival.—It is of interest to know whether the larvae produced by a given mating showed about the same survival as the larvae from another mating within the same line. This question is investigated by the analysis in table 3. There are no significant chi-squares. It is concluded that all matings within lines showed similarity in survival under each type of treatment. The matings within lines are pooled for further analysis.

Spore and check treatments showed differences in survival.—A glance at the data suggests that there are real differences in survival following the two treatments. A formal comparison of treatments is made in table 4. The chi-squares are very highly significant. In less than one out of 10,000 samples would one expect deviations so great if spore and check treatments gave the same results. It is concluded that spore treatment led to decreased survival in the case of each line.

The pooled per cent survivals following the two treatments in the three lines are given in table 4. In each line, check-treatment survival was in excess of 95 %. Survival following spore treatment was much lower, and there was a difference of 20% or more between lines.

Lines showed differences in survival.—The most important question in this investigation is whether the three lines showed different survival under similar treatment. Table 5 shows that for the checks there is no evidence against the hypothesis that the Van Scoy and Brown lines survived to the same extent. The same holds for Van Scoy and chartreuse. The difference between Brown and chartreuse might be considered as significant but it is so small as to be of little importance in this investigation.

In contrast, large differences between lines appear when spore treatments are considered. The chi-squares are highly significant and the P values exceedingly small. Consequently, it is concluded that larvae of the three lines of bees showed different survival following exposure to the pathogen. This is interpreted to be an expression of a real difference in susceptibility.

DISCUSSION.—The results presented diverge from those presented by Woodrow (1942). Woodrow concluded, "There was no evidence of larval resistance to the disease. . . ." The technique of individual inoculation used in the present work was essentially the same as the one developed and used by Woodrow. The great difference between his work and ours lies in the bees used. Each line used in this investigation had been bred for resistance or susceptibility by the use of artificial insemination. No mention is made of a susceptible line specifically selected and bred for susceptibility in Woodrow's paper nor indeed in any paper on the subject of resistance to American foulbrood known to us. Artificial insemination of bees now gives much greater control over line development than could be obtained at the time of Woodrow's work (Mackensen & Roberts 1952). Even if the susceptible stock used by Woodrow was very susceptible and very greatly differentiated from his resistant stock, the two stocks obviously were not differentiated with respect to larval resistance.

Beyond the fact of different lines of bees in the two investigations are the possible differences in the strains of pathogen used. These may or may not be responsible for some of the differences in results.

With this demonstration of different levels of resistance in larvae of different lines of bees, certain new problems arise regarding the physiological and hereditary bases of larval resistance. A great deal of work has been done on the question of the physiological nature of resistance and immunity in other insects (Steinhaus, 1949). Only a small start has been made on the honey bee-foulbrood relationships. Gary *et al.* (1948) reported, in a preliminary study, a higher concentration of agglutinins of *Bacillus larvae* in the blood of adult bees than in the blood of rather large larvae. Whether immune bodies in the blood have any relationship to the resistance in these very small larvae is an open question. It is not considered likely that the apparent larval resistance in our lines could be due to the

Table 3.—Comparisons of matings within lines and treatments, pooling data from both experiments.

LINE	TREATMENT	MATING	SURVIVORS	NON-SURVIVORS	CHI-SQUARE	P
Van Scoy	Check	M 806	109	1		
	Check	M 808	127	6	1.6557	.10–.20
	Spore	M 806	44	109		
	Spore	M 808	24	99	2.6572	.10–.20
Brown	Check	M 899	106	3		
	Check	M 926	224	2	0.6994	.30–.50
	Spore	M 899	93	54		
	Spore	M 926	158	69	1.3521	.20–.30
Chartreuse	Check	M 869	122	5		
	Check	M 872	110	5		
	Check	M 876	152	8	0.1953	.90–.95
	Spore	M 869	73	73		
	Spore	M 872	46	57		
	Spore	M 876	67	79	0.8259	.50–.70

Table 4.—Comparisons of treatments within lines, pooling data from the two or three matings and both experiments.

LINE	TREATMENT	SURVIVORS	NON-SURVIVORS	CHI-SQUARE	P	PER CENT SURVIVORS
Van Scoy	Check	236	7			97.1
	Spore	68	208	277.0	<0.0001	24.6
Brown	Check	330	5			98.5
	Spore	251	123	115.7	<.0001	67.1
Chartreuse	Check	384	18			95.5
	Spore	186	209	227.1	<.0001	47.1

Table 5.—Comparisons of lines within treatments, pooling data from the two or three matings and both experiments.

TREATMENT	LINE	SURVIVORS	NON-SURVIVORS	CHI-SQUARE	P
Check	Van Scoy	236	7	0.7876	.30–.50
	Brown	330	5		
	Van Scoy	236	7		
	Chartreuse	384	18	0.6546	.30–.50
	Brown	330	5		
	Chartreuse	384	18	4.4354	.02–.05
Spore	Van Scoy	68	208		
	Brown	251	123	112.9	<.0001
	Van Scoy	68	208		
	Chartreuse	186	209	33.87	<.0001
	Brown	251	123		
	Chartreuse	186	209	30.59	<.0001

activity of bacteriophage (Gochnauer 1955). Nevertheless, the possibility has not been ignored in the plans for further work, and experiments now underway will provide evidence on the question. The hereditary basis of the resistance is an equally important problem, and here again, much work has been done on other species (Gowen 1948, 1954). If larval resistance is a desirable characteristic for incorporation into a better bee, the heredity of the resistance is an urgent, practical problem. It is an important fundamental problem regardless of economic value of the characteristic.

As related in the materials and methods section, the queens of the chartreuse line which were used in this investigation had survived massive inoculation of their larval food with spores of *Bacillus larvae*. This procedure was designed and utilized by us in an effort to select specifically for what was then a hypothetical larval resistance. It was supposed that such selection applied to both queens and drones over a series of generations could raise the level of larval resistance sufficiently for it to be recognized easily, especially when contrasted with that of a line selected for susceptibility. Now that larval resistance has been demonstrated, experiments are underway to evaluate the effectiveness of the individual inoculation-selection procedure in altering its level.

Physiological differences in different strains of *Bacillus larvae* have been noted by various workers for many years. Is this resistance in larvae effective against all strains of the pathogen?

A large amount of work has been done on the use of drugs to control American foulbrood. The possible effect of variability in the pathogen on successful use of drugs has been pointed out repeatedly. Now it must be inquired what effect variable resistance in the host larvae have upon results of chemotherapeutic experiments of different investigators using different stocks of bees.

As discussed in the introduction, several mechanisms of resistance to American foulbrood in honey bees now are known. Is any one of these mechanisms sufficient by itself to confer resistance upon a colony? The part each mechanism of resistance plays in overall colony resistance when several mechanisms are operating simultaneously is a matter of importance. It is important also to know the hereditary basis of the transmission of each mechanism from parent to offspring. For it is only after the various mechanisms of resistance have been defined as to their relative roles and genetic bases that it becomes easily possible to synthesize stocks for new experiments or for practical utilization of resistance.

SUMMARY.—Larvae of three genetically distinct lines of honey bees were compared as to survival following individual inoculations of larval food with 50,000 spores of *Bacillus larvae* in water. Larval age in each test comb varied less than 24 hours, inoculations were made when larvae were less than 24 hours of age, and all test combs were placed in the same colony for brood rearing, on the same day, immediately after the eggs were laid. Check larvae, given water containing no spores, showed survival in excess of 95% in each line. Spore-treated larvae survived to the extent of 25% in a line selected and bred for susceptibility, 47% in a line selected for larval resistance for one generation, and 67% in a line selected and bred for resistance. Differences between the lines in survival of spore-treated larvae are highly significant. These differences are interpreted to be due to different levels of innate resistance to American foulbrood in the larvae of the three lines.

REFERENCES CITED

Gary, N. D., C. I. Nelson, and J. A .Munro. 1948. Serological evidence of resistance of larvae and workers to *Bacillus larvae*. Jour. Econ. Ent. **41**(4): 661–3.

Gochnauer, T. A. 1955. The isolation of a bacteriophage (bacterial virus) from *Bacillus larvae*. Bee World **36**: 101–3.

Gowen, John W. 1948. Inheritance of immunity in animals. Ann. Rev. Microbiol. **2**: 215–54.

Gowen, John W. 1954. Significance and utilization of animal individuality in disease research. Jour. Nat. Cancer Inst. **15**: 555–70.

Mackensen, Otto, and William C. Roberts. 1952. Breeding Bees. Insects, The Yearbook of Agriculture. U. S. Dept. Agr. Washington, D. C.

Park, O. W. 1936. Disease resistance and American foulbrood. Amer. Bee Jour. **76**: 12–15.

Park, O. W. 1937. Testing for resistance to American foulbrood in honeybees. Jour. Econ. Ent. **30**(3): 504–12.

Park, O. W. 1942. Variation in resistance to American foulbrood in honeybees. Report on Agricultural Research. Agr. Expt. Sta., Iowa State College. Part I: 176–7.

Park, O. W., Frank C. Pellett, and F. B. Paddock. 1937. Disease resistance and American foulbrood. Amer. Bee Jour. **77**: 20–5.

Rothenbuhler, Walter C., John W. Gowen and O. W. Park. 1953. Allelic and linkage relationships of five mutant genes in honey bees (*Apis mellifera* L.) Jour. Hered. **44**: 251–3.

Steinhaus, Edward A. 1949. Principles of insect pathology. 757 pp. McGraw-Hill Book Company.

Sturtevant, A. P., and I. L. Revell. 1954. Reduction of *Bacillus larvae* spores in liquid food of honey bees by action of the honey stopper, and its relation to the development of American foulbrood. Jour. Econ. Ent. **46**(5): 855–60.

Tarr, H. L. A. 1937. Studies on American foulbrood of bees. I. The relative pathogenicity of vegetative cells and endospores of **Bacillus larvae** for the brood of the bee. Ann. Appl. Biol. **24**: 377–84.

Thompson, Victor C. 1955. A new type of resistance to American foulbrood originating in the nurse bees. Unpublished M.S. thesis. Iowa State College, Ames.

Woodrow, A. W. 1941a. Susceptibility of honeybee larvae to American foulbrood. Gleanings in Bee Cult. **69**: 148–51.

Woodrow, A. W. 1941b. Behavior of honeybees toward brood infected with American foulbrood. Amer. Bee Jour. **81**: 363–6.

Woodrow, A. W. 1942. Susceptibility of honeybee larvae to individual inoculations with spores of *Bacillus larvae* Jour. Econ. Ent. **35**(6): 892–5.

Woodrow, A. W., and H. J. States, Jr. 1943. Removal of diseased brood in colonies infected with A.F.B. Amer. Bee Jour. **83**: 22–3.

Woodrow, A. W., and E. C. Holst. 1942. The mechanism of colony resistance to American foulbrood. Jour. Econ. Ent. **35**(3): 327–30.

Mass Rearing and Testing Techniques for *Typhlodromus fallacis* (Gar.)[1]

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Predaceous mites are important animals in the intricate scheme of natural control. DDT and many other organic pesticides have focused more attention on the importance of these predaceous arachnids. Consequently many more entomologists have organized long range programs to measure the importance of a multitude of pesticides on the population fluctuations of predatory mites.

Predaceous mites are not only small but they move rapidly, therefore laborious sampling techniques are needed to evaluate pesticidal toxicity and to glean biological information. Obviously if these mites could be reared continuously in the greenhouse, not only could we study their biology but we could also gain a quick insight into the complex picture of the mite's tolerance to the numerous fungicides, acaricides and insecticides.

By chance, but also by good fortune, the author in 1951 isolated a small colony of *Typhlodromus fallacis* from a depleted population of two-spotted spider mite, *Tetranychus telarius* (L.), in the greenhouse. Several months later, studies were initiated on the mass rearing techniques, and techniques for measuring the toxic action of several pesticides to the mite. In the spring of 1952, a satisfactory mass rearing method was developed. Subsequently Ballard (1953), Daugherty (1953) and Stevenson (1955) modified the technique to study the life history and to accumulate information on the influence of pesticides on this important phytoseiid mite.

Method A.—The two-spotted spider mite was cultured to feed *T. fallacis*, therefore the author believes this method should also be described. The two-spotted spider mite was reared on red kidney bean plants. These beans were planted in a mixture of sterilized sand and vermiculite (3:1) in large flats (21 by 15 by 3 inches). Eighty seeds (seven-eighths of a 50-ml. beaker) were planted per row and four rows to a flat. When the primary leaves were 3 to 5 days old, trifoliate leaves were taken from the stock flat and distributed over this new foliage. About seven leaflets, containing about 50 adult two-spotted spider mites were spread over each row of plants. In the summer this number will suffice but in the fall and winter another complement may be needed the next day, especially if the stock flat is not heavily populated. These dried leaflets should be removed the third day to insure a more uniform population. This sytem of 7 to 14 leaflets per 21-inch row is geared to forestall over and under population and to insure a high population of adult mites on the second set of trifoliates in 10 days.

A series of four flats about 10 days apart will provide a population of *telarius* large enough to maintain at least three flats of the predator.

Beans growing in the greenhouse in the fall and winter will need supplementary light, especially during cloudy weather. Our growing benches were provided with adjustable units of two 90-w. daylight fluorescent tubes placed 24 inches above the height of the plants. Vigoro was sprinkled over each furrow at one heaping teaspoon per furrow. The rule of tumb is to use plants which are not too young (less than 3 days) or too old (older than 7 days), because in the former choice mites tend to overpopulate the flat of beans and if the plant is too old mite populations are too diluted.

Method B.—There are no references to the mass rearing of phytoseiid mites, but Herbert (1952) and Huffaker (1953) describe methods of rearing small populations in the laboratory or in the greenhouse. The method described in this paper pertains to *T. fallacis* but undoubtedly it is applicable to other phytoseiid mites.

Colonies of *fallacis* were maintained for 3½ years in the greenhouse where daily temperatures fluctuated but night temperatures were maintained at 78°F. The greenhouse was shaded lightly with a soya bean-lime mixture, from June through September.

Usually arthropods are not easily reared until we learn more about their habits and their environmental requirements. This typhlodromid mite is no exception. However, its abundance is most seriously controlled by only one factor—an inexhaustible supply of two-spotted spider mites. Stevenson (1955) has shown that *fallacis* will even oviposit at 50° F. The colonies were cultured on red kidney beans, or the Henderson bush limas. The beans are planted similarly to those described under the section for the two-spotted spider mite.

The plants for the new predaceous mite cultures should be younger. The plants should be infested with the two-spotted spider mite when all the primary leaves are only 1 to 2 days old. The host doesn't have sufficient time to overpopulate the leaf and thus force migration if older plants are used for the cultures. Mites move from the overpopulated or the chlorotic primary leaves to the trifoliate leaves.

Each row of beans should be infested with a minimum of 15 leaflets. Three days later the predaceous mites are introduced. These transfer leaflets should not contain more than 10 adult mites and if the flat will be used for testing, each plant should be infested. If the flat will not be used for a pesticide series first, 20 evenly distributed leaflets per row provide a uniform population. The neophyte tends to overpopulate the stock cultures with the predatory mite.

In a balanaced series, the predaceous mites move to the trifoliate leaves after the phytophagous mite. Populations on the new foliage should be checked under the binocular. Frequently more food must be added to support a high population. According to Ballard (1954) *T. fallacis* consumes four to eight eggs or the motile forms of the two-spotted spider mite. A series of five flats at about 10-day intervals will provide enough mites for any large pesticide evaluation program.

Old stock cultures should be killed by steam in a 30-gallon garbage can which has a hole cut in the lid for the steam hose. This sanitary measure must be practiced if the two-spotted spider mite is to be maintained at peak

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population. Even with these sanitation methods, the host mite colony must be checked periodically. If *fallacis* is found in the stock colony, (this is the rule, not the exception) the plants should be sprayed twice with a 500-p. p. m. DDT emulsion, 7 days apart.

TESTING METHODS.—The adult predaceous mite moves rapidly and doesn't lend itself to conventional confinement techniques. Ballard (1953) used a modified Huffaker cell to confine *T. fallacis*, Herbert (1952) placed mites in vials. These methods, however, have limited applications and are tedious. We developed (Ristich 1953) a technique for keeping many mites in a small area using Henderson lima beans placed in a 9-cm. petriplate which contained three discs of filter paper moistened with 3 ml. of distilled water, (Fig. 1). Tanglefoot or Stop was applied with an inexpensive cake decorator (Fig. 2) to confine the mites. This technique can be used for gathering biological data Stevenson (1955) or for evaluating pesticides. Stop is more useful than Tanglefoot since it is less phytotoxic to some varieties of beans (red kidney) and because the surface doesn't harden as quickly.

A detailed description of this method and our older methods of evaluating pesticides against predaceous mites is included in the following paragraphs.

Henderson bush lima beans or red kidney beans are planted in 2½-inch pots (two per pot). The lima beans are more desirable because (1) the under surface is not hairy, (2) the veins are not too prominent,[3] (3) the lima bean leaves are more tolerant of subdued light and necrotic areas do not develop as rapidly, as in the kidney bean, when Tanglefoot is used. These advantages, to the investigator, outweigh the disadvantages of slower germination, slower growth and the additional cost.

Three to five days after the primary leaves expand, the plants are ready for the experiment. Before the mites are

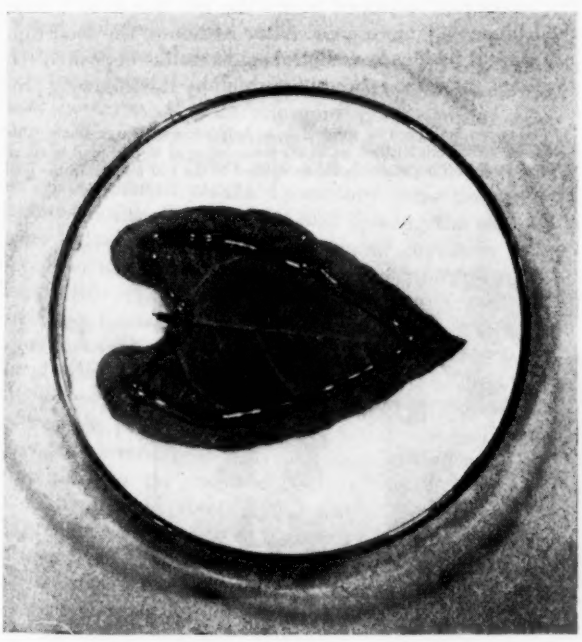

FIG. 1.—A Henderson lima bean leaf ringed with Tanglefoot within a petri plate containing three discs of moist filter paper.

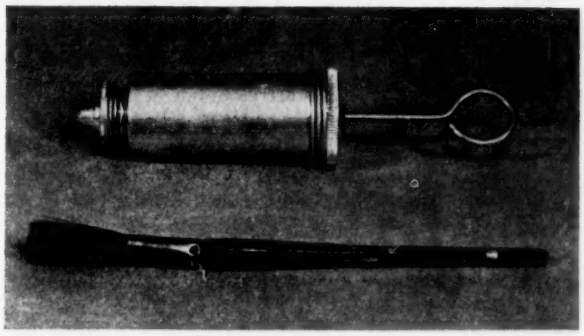

FIG. 2.—An inexpensive cake decorator for applying Tanglefoot or Stop and a stiff brush for brushing *Tetranychus telarius* onto treated leaves.

added, the leaves can be trimmed with a leaf shaper Cole (1955) or with scissors. Younger leaves can be used uncut. Next, the plants are infested with the two-spotted spider mites from the stock culture. One leaflet with about 50 mites is placed on each primary leaf. The pots are placed in long water trays. Predaceous mites are added the next day. Ten to twenty mites on one leaf will produce 20 to 50 eggs per day. A large population of mites on a small leaf will "cluster" the eggs, table 1. This bunching habit

Table 1.—Egg distribution on lima bean leaves by *Typhlodromus fallacis*.

SERIES	NO. OF EGGS ON LEAF		PER CENT OF TOTAL ON BASAL HALF	NO. OF EGGS IN CLUSTER
	Basal Half	Apex Half		
A	35	2	95	8
B	15	2	89	0
C	26	6	80	4, 4, 4
D	29	0	100	11
E	25	5	85	11
F	33	0	100	15
G	27	8	77	13
H	17	14	56	0
I	9	15	37	0
J	9	26	26	5

reduces the efficiency of the test. When predaceous mite populations are low, several transfers should be made to the test leaf in 1 day, with small leaf sections. In the summer, eggs will hatch in 36 hours.

Previous methods for evaluating pesticides against *fallacis* were unsatisfactory. Ristich (1952) and Daugherty (1953) dipped plants in the formulation; and placed the cut stems in a small gas bottle containing water and a cellucotton plug. This container was placed on an 8-inch disc of wax paper. The wax paper was ringed with Tanglefoot. Data were recorded 24 or 48 hours later. Live and dead mites were counted on the leaves; then the wax paper was placed over a cardboard grid and live and dead mites were counted on alternate grids—similar to the Henderson-McBurnie European red mite tech-

[3] Mites deposit many eggs along the midrib. The experimenter loses much time scrutinizing the leaf for the partially hidden oval eggs.

Table 2.—An evaluation of the Tanglefoot-petri dish method for confining *Typhlodromus fallacis*. 1954.

Series	Egg Counts[a]	No. of Live Mites After 3 Days	Per Cent Recovery
A	73	60	82
B	116	113	97
C	164	164	100
D	96	83	85
E	49	56	100+[b]
F	73	67	91
G	197	240	100+[b]
H	159	141	88

[a] Average of three replicates.
[b] Some eggs probably hidden along midvein.

nique. This was a fair screening method but too cumbersome and erratic.

Subsequently, leaves were trimmed and dipped (Fig. 3), or sprayed and placed in plastic cup units, Lea (1954) with organdy covered lids. These leaves could be placed in a counting frame (Fig. 4) where the rapidly moving mites can be counted quickly. The live and dead mites are then quickly counted on the bottom of the plastic cup by placing the cup over a colored sectioned disc. This method was less cumbersome but mites moved off the leaf onto the sides of the cup. Fair estimates can be made on residual toxicity.

In the present petri dish method, the leaves are trimmed or untrimmed and dipped (Fig. 3) or sprayed with a 50-p. p. m. TEPP solution containing Ultrawet (100 p. p. m.).

The TEPP kills all the motile phytophagous and predaceous mites. After the water evaporates, these plants are treated with the pesticide.[4] Captan at 500 p. p. m. will reduce fungal growth on the leaf and on the Tanglefoot or Stop.

After the treatment, the petiole is cut near the base of the leaf (Fig. 1) and a barrier of thin Tanglefoot is placed along the margin with a small cake decorator. When the *Stop* is stored in a cool place, it will not bond completely at the base of the midvein. This area can be compressed lightly with a toothpick, otherwise larvae or nymphs will crawl through or hide in the tiny niche. The leaf is then

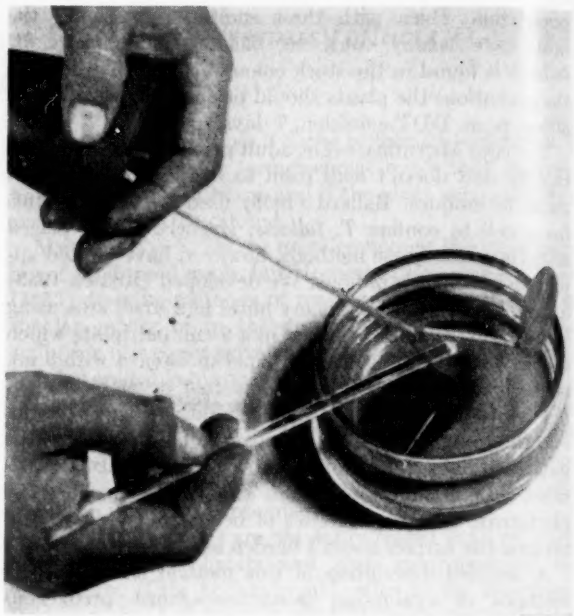

FIG. 3.—One method of evaluating pesticides against mites; note trimmed leaves.

placed in the petri dish[5] which contains three discs of 9-cm. filter paper moistened with 3 ml. of distilled water. These discs will remain moist for 48 to 72 hours. Water can be pipetted onto the discs as the water evaporates. These petri plates are kept under two 90-w. daylight fluorescent lights. Stevenson (1955) has kept these lima bean leaves for 13 days at 55° F. in total darkness without leaf chlorosis.

Data can be recorded easily if the cover is removed and the lower unit is placed under the low power of a wide field binocular microscope. After 24 hours, the food supply should be checked. If the spider mites deposited too few eggs, more food should be added by the following pro-

[4] Plants can be dipped or sprayed in a Jeffers-Ristich Spray Booth (Cole (1955) for 45 seconds with 90 ml. of the formulation at 40 p.s.i. and 30 r.p.m.
[5] The Stop or Tanglefoot should be applied to the leaf outside of the petri plate.

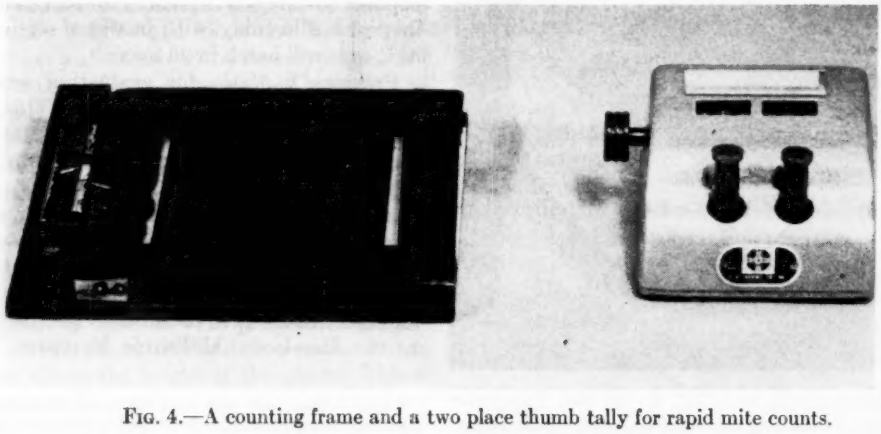

FIG. 4.—A counting frame and a two place thumb tally for rapid mite counts.

cedures. Leaflets are plucked from the stock flats. The mites and eggs are carefully brushed onto the test leaves with a moderately stiff brush (Fig. 2). When the leaflet is held close to the test leaf, the mites and the eggs can be brushed into the barrier without impaling too many on the Stop. In the other method, leaflets from the stock culture are placed in a large Erlenmeyer flask with strips of wax paper. A lamp is placed over the flask. As the leaflets dry the mites crawl onto the wax paper or the bottle. These can be brushed into the barrier more easily.

The present method, obviously, has its limitations also, especially if the experimenter is interested in securing rapid contact toxicity data to adults. The problem, however, is not insurmountable since the leaves can be sprayed in a Barnhardt Tower after the barrier is placed around the margin.

SUMMARY.—A method is described for mass rearing of the two-spotted spider mite, and the predaceous mite *Typhlodromus fallacis*. Several methods for evaluating pesticides against this predaceous mite are described and evaluated.

REFERENCES CITED

Ballard, R. C. 1953. The biology of the predacious mite *Typhlodromus fallacis* (Gar.) at 78° F. Master's Thesis, Ohio State University.

Ballard, R. C. 1953. A modified Huffaker cell. In Peterson's Manual of Entomological Techniques (Rev.). Plate 153.

Ballard, R. C. 1954. The biology of the predacious mite *Typhlodromus fallacis* (Gar.) at 78° F. Ohio Jour. Sci. **54:** 175–9.

Cole, C., and F. W. Fisk. 1955. Comparative toxicity of certain acaricides to the carmine and green forms of the two-spotted spider mite. Jour. Econ. Ent. **48:** 85–87.

Daugherty, D. 1953. Partial survey of the insecticidal tolerance of the predacious mite *Typhlodromus fallacis* (Gar.). Master's Thesis, Ohio State University.

Herbert, H. J. 1952. Progress report on predacious mite investigations in Nova Scotia. Ann. Rpt. Ent. Soc. Ont. **83:** 27–9.

Huffaker, C. B., and C. E. Kennett, 1953. Differential tolerance to parathion of two *Typhlodromus* predatory on cyclamen mite. Jour. Econ. Ent. **46:** 707–8.

Huffaker, C. B., and C. E. Kennett, 1953. Development toward biological control of cyclamen mite on strawberries in California. Jour. Econ. Ent. **46:** 802–12.

Lea, A. 1954. Plastic dishes in the entomology laboratory. Turtox News. **31:** 8.

Ristich, S. S. 1952. A technique for evaluating pesticides against the predaceous mite *Typhlodromus fallacis* (Gar.). Unpublished.

Stevenson, J. O. 1955. The toxicity of several chemicals to the predaceous mite *Typhlodromus fallacis* (Gar.) and notes on its biology. Master's Thesis, Ohio State University.

Wireworm Control in Iowa Sweet Potato Fields[1]

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Sweet potatoes grown under irrigation in the coarse sandy soil of the Muscatine Island area of Iowa are relatively free of pests which attack the aerial portions of the plant; however, the underground portions are often severely injured by wireworms of the genus *Melanotus*. These wireworms burrow into and hollow the stems of newly transplanted plants. Later they enter the edible portions causing disfigurement and opening the way for disease inducing organisms. Preliminary counts in 1949 showed an average of 52% of the sweet potatoes produced in this region were injured by wireworms.

Griffin & Eden (1953) have shown that control of wireworms in sweet potatoes is practical. They noted good control with lindane, heptachlor, aldrin, dieldrin and BHC when these materials were applied with the fertilizer 2 weeks previous to planting. Chlordane and toxaphene gave erratic results while parathion and ethylene dibromide gave no practical control. No off-flavors or reductions in yield were noted in any of these tests.

METHODS.—In wireworm control tests in the Muscatine area from 1950 through 1954, the efficiency of various insecticides, methods for their application, and dosages were investigated. In all tests, plots were each six rows wide and 20 feet long, 1/20-acre in size. Each treatment was replicated six times in a randomized design. At harvesting time the middle two rows were dug, sorted to number one and number two roots and then further sorted into wireworm-damaged and undamaged, weighed and counted.

1950.—The six wettable powder insecticides listed in table 1 were mixed with water and poured about the plant roots when the slips were transplanted to the field. Perthane and chlordane were used at 1 pound actual toxicant per acre and the other materials at 0.5 pound.

1951.—In 1951 the efficiency of insecticides applied in the transplant water and in the fertilizer was tested. Slurries were made of the wettable powders and added to the transplant water. Wettable powders were also thoroughly mixed with the fertilizer and this material applied to the plots in a row application. Aldrin and dieldrin were used at 0.5 pound per acre and the chlordane at 1 pound.

1952.—Aldrin, which had appeared so promising in 1951, was applied in the transplant water at 0.5 pound per acre to areas in five fields in the Muscatine area. These plots were harvested separately and yield and damage were compared with that from a similar area on either side of the treated plot. Two soil fumigants were tested by opening the planting row 6 to 7 inches deep

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Table 1.—Effect of insecticide wettable powders on yield and wireworm damage of sweet potatoes. Fruitland, Iowa, 1950.

Treatment	Pounds Toxicant per Acre	Yield (Lbs.)	Number Damaged[a]
Perthane	1	12.8	1.57
EPN	0.5	12.4	2.17
Chlordane	1	12.5	1.14
Aldrin	0.5	11.6	1.18
Dieldrin	0.5	13.8	1.26
Lindane	0.5	12.7	0.92
Check		13.1	2.59
		L.S.D. at 5% level	0.83
		at 1% level	0.44

[a] 1 Converted to $\sqrt{x+.5}$ units.

and applying 0.5 pint of ethylene dibromide per 100 feet of row and 1 quart of D-D mixture per 340 feet of row. These materials were then covered over and the sweet potatoes transplanted 1 month later.

1953 and 1954.—Since previous tests had shown that aldrin gave excellent control of wireworms yet caused some reduction in yield, tests were made in 1953 of various dosages of aldrin emulsions and wettable powders. In 1954 further dilutions were tested using only the emulsion form. Heptachlor and endrin were also included in the latter test. All of these were applied in the transplant water.

Results.—In 1950 all chemicals tested except EPN significantly reduced the number of wireworm-damaged roots. However, a lower yield was obtained with each of the materials, except dieldrin, than from the untreated check. (Table 1).

The insecticides applied in the transplant water were much more efficient in controlling wireworm damage than the same chemicals applied with the fertilizer, but there were no differences in the yield from these two treatments (Table 2). The reason for this was probably due to the better distribution of the insecticide in the soil when applied by water.

In 1952 where aldrin was used at 0.5 pound per acre, a yield of 16.3 pounds per plot resulted, while the untreated plots yielded 19.5 pounds. However, the untreated plots had 183 damaged potatoes, whereas in the aldrin plots only six damaged potatoes were found.

Table 2.—The effect of insecticides when applied in transplant water or in fertilizer mixtures on yield and wireworm damage of sweet potatoes. Fruitland, Iowa, 1951.

Treatment	Yield (Lbs.)	Number Damaged
Transplant water		
Aldrin	16.0	0
Dieldrin	19.2	11
Chlordane	17.2	6
Fertilizer mixture		
Aldrin	17.4	79
Dieldrin	18.7	42
Chlordane	14.2	61
Check	13.3	101

Table 3.—Effect of various dosages of aldrin wettable powders and emulsions applied in transplant water on yield and wireworm damage of sweet potatoes. Fruitland, Iowa, 1953.

Pounds Actual Toxicant per (Acre)	Yield (Lbs.)	Number Damaged
Wettable powder		
0.25	15.6	0
0.5	15.8	5
1	14.0	0
Emulsion		
0.25	15.1	2
0.5	13.9	0
1	13.1	0
Check	19.5	63
L.S.D. at 5% level	0.92	
at 1% level	1.24	

Despite the obvious lower yield, the growers felt the treatment desirable, since the potatoes were practically free of wireworm damage.

Under the conditions of these experiments, the soil fumigants seemingly did not show promise for practical control. Ethylene dibromide had no affect on yield and reduced wireworm damage by only 12%. The D-D mixture reduced the number of damaged roots by 33% but seriously reduced yield. Seventeen pounds were produced in the check plots and only 14.4 pounds in the treated plots.

The dosages used in 1953 were still too high to prevent the reduction in yield but good control was still obtained at the lower dosages. There seemed to be no difference in the performance of wettable powders and emulsions in these tests, except that emulsions tended to stay in solution longer than the wettable powders which tended to settle out in the unagitated transplant water. The reductions in yield were highly significant for all treatments (Table 3).

In 1954 treatments significantly increased yields and yet gave good wireworm control (Table 4). Aldrin emul-

Table 4.—Effect of insecticide emulsifiable concentrates applied in transplant water on yield and wireworm damage of sweet potatoes. Fruitland, Iowa, 1954.

Materials and Pounds Actual Toxicant per Acre	Yield (Lbs.)	Number Damaged
Aldrin		
0.125	17.5	2
0.25	15.8	0
0.5	15.1	0
Heptachlor		
0.25	15.3	0
0.5	15.5	0
Endrin		
0.25	17.6	0
0.5	14.4	3
Check	16.8	60
L.S.D. at 5% level	0.71	
at 1% level	1.86	

sifiable concentrate at 0.125 pound (actual) per acre or endrin at 0.25 pound per acre applied in the transplant water resulted in an increase in yield and still held the wireworm damage to a low level.

SUMMARY.—Sweet potatoes grown in the Muscatine Island area of Iowa are often severely injured by wireworms of the genus *Melanotus*. Control tests were made in this area from 1950 through 1954 with investigations of the efficiency of various insecticides in the soil, methods for their application, and dosage. Insecticides applied in the transplant water gave better control than the same materials applied to the rows in a fertilizer mixture. Aldrin at 0.125 pound per acre gave satisfactory control and avoided the suppression of yield which it caused at higher dosages. Soil fumigants gave no practical control.

REFERENCE CITED

Griffin, J. A., and W. G. Eden. 1953. Control of the gulf wireworm in sweet potatoes in Alabama. Jour. Econ. Ent. **46**(6): 948–51.

Package Exposure to Continuously Vaporized Lindane[1]

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Packaged foods held in storerooms, warehouses and other storage areas are often attacked by stored food insects since package materials do not necessarily protect the contents from insect invasion (Essig *et al.* 1943, Gerhardt & Lindgren 1954). As is common with many storage areas, structural pests such as house flies and cockroaches may also be present. Continuous vaporization of lindane has been employed as a control measure against these pests. With this method of control any insects gaining entrance from infested quarters nearby, or from previously infested foods or packaging materials are usually eliminated before new infestations are established.

Many food packages function merely as food containers. However, others may be constructed to permit the exchange or the exclusion of gases or vapors. Queen (1953) has reported that package pentration by lindane vapor at high concentrations does occur with some packaging materials.

PROCEDURE.—The object of this study was to determine if contamination of packaged foods occurred upon exposure to an atmosphere of lindane, as well as the relative quantities of lindane vapor penetrating, and the type of package that is most susceptible to lindane vapor penetration. The lindane was vaporized at the commercially recommended rate of approximately 1 gram of lindane per 15,000 cu. ft. per 24 hours.

The test site was a basement food storage area. The walls and floor were of finished concrete. There were seven windows and two doors. The windows (15×24″) were placed at ceiling height. The ceiling consisted of studding with a board floor which was surfaced with a floor covering. During the period of exposure two or three windows remained open continuously. One door opened to the outside, serving as a loading and unloading entrance and the other door opened to a corridor leading to a kitchen area. These doors usually remained closed. The volume of treated space was 33,687 cu. ft.

Two vaporizers[2] were installed, one near each end of the room. The vaporizers were placed so that their position did not interfere with the usual movement of stored materials. The average rate of vaporization was about 2.05 grams per 24 hours or about 1 gram per 24 hours per 16,240 cu. ft. The approximate average vapor concentration was 0.038 micrograms per liter.

The foods to be exposed were placed on the second shelf from the top of a typical storage rack. A distance of about 2 inches was maintained between packages. Samples were taken after varying periods of exposure. All food samples were analysed for lindane by the Schechter-Hornstein method (1952). Air samples were taken according to the procedure of Hornstein & Sullivan (1953). In nearly all cases samples were analysed immediately after being collected. Samples not analysed immediately were stored in glacial acetic acid under refrigeration.

The foods were purchased at a neighborhood store and were not previously exposed to lindane vapor, in so far as is known. A representative sample of each food as purchased was analysed. The results presented in the tables have been corrected accordingly. The foods and their packaging materials were as follows:

1. Cornflakes, Kellogs, 8-oz. pkg. The package was composed of two parts, an inner sack of 25-pound waxed glassine and an outer box of White Patent Newsboard having a thickness of 0.0175 inches. The dimensions of the package were 17.5×24.5×6 cm. The sample was taken by carefully slitting through both packages, pouring the contents into a mortar and reducing the cornflakes with a pestle. The sample was divided into two flasks, run separately and then the m-dinitrobenzene extracts were combined and determined colorimetrically as one sample.

2. Shredded Wheat Biscuits, Nabisco. The box was coated White Patent Newsboard with a caliper of 0.020 inches. The package size was the 6-oz., "New Small Family Package." The dimensions of the package were 10.8×7.7×19 cm. Samples were taken from the top layer. The biscuits were reduced in the same manner as the cornflakes.

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[2] An Aerovap, supplied by American Aerovap, Inc., New York City.

3. Pastry Mix, Flako, standard 9-ox. size package. The dimensions were 7×14×3.5 cm. The packaging materials were of two sections, an inner bag consisting of a laminated liner paper, 47-pound basis, and an outer carton of 0.020 coated White Patent Newsboard. Samples were taken from the top of the package. The sample for each package was divided into two flasks and analysed separately. The m-dinitrobenzene extracts in ether were combined and determined colorimetrically as one sample.

4. Flour, Pillsbury's Best, 2-pound package. The bag was of uncoated, unlined bleached kraft paper. The dimensions of the package were 11×7×16 cm. The samples were taken in the following manner. The package was carefully placed horizontally on the two longest dimensions so as not to allow the flour to flow. The top (side) 1 cm. layer of flour was carefully removed; then a 2-cm. layer was removed and finally a section 3.7 and 2 cm. from the bag wall (center) was sampled.

When analysed the untreated flour samples were variable in their apparent interference content; therefore, an average of eight samples was taken as the mean interference. The untreated samples varied ±0.3 p.p.m. from the mean. It is surmised that the variations in results were due to various degrees of charring in the samples during analysis.

5. Soup, A Dehydrated Mix—Lipton's Chicken Noodle. The outer package was of boxboard with dimensions of 12.7×10.5×4 cm. There were three separate inner packages (13.2×10.6 cm.) of a laminated aluminum foil (aluminum to paper). Each inner package contained 2⅛ oz. dry soup mix. The total sample taken for analysis consisted of two inner envelopes.

6. Shredded Coconut, Lake Shore Brand, 2-oz. package. The package consisted of two cellophane bags, one within the other, heat sealed at the top and cemented at the base. The samples were taken from the top and bottom of the package or a representative sample was taken after thoroughly mixing the contents.

DISCUSSION.—The corn flakes package showed no appreciable passage of lindane vapor as indicated in table 1.

Results of 24.4 p.p.m. at the top of the shredded wheat package and 8.3 p.p.m. at the bottom indicated that penetration was greatest at the top of the package. Perhaps this is a result of having the bottom of the package resting on the shelf and thus not permitting an equal penetration of the surfaces both at the top and bottom. The total surface area of the package minus the bottom area is 785 sq. cm.; the bottom area is 83 sq.cm. If it follows that non-penetration through the bottom areas was the primary cause of the stratification of the lindane concentration in the package, then the lower half of the package should have a concentration of approximately 21% less than the top half of the package. The actual difference is almost twice the estimated concentration. Thus the lower concentration at the bottom may have been due, partly, to the decreased surface offered by the bottom half of the package and partly due to the top flap being not as well sealed. This latter factor was observed in many of the packages. The packages often exhibited a variable top seal while the bottom seal was more uniform.

The rate of sorption exhibited by the exposed un-

Table 1.—Package exposure to continuously vaporized lindane.[a]

Sample No.	Days Exposed	Position in Sample in Pacakge	Lindane Found: P.p.m.	Lindane Found: P.p.m. per Day
		1. Corn flakes		
1	52	top	0.0	0.0
2	52	top	.0	.0
3	106	top	.3	.00283
		Mean p.p.m./day		*.00094*
		2. Shredded wheat		
1	33	top	4.6	0.140
2	33	top	5.8	.175
3	104	top	21.4	.203
		bottom	8.3	.080[b,c]
4	104	top	20.2	.194
		Mean p.p.m./day		*.178*
		Shredded wheat, unpackaged		
1	7		6.7	
2	7		13.5	1.50
3	7		11.4	
		Mean	*10.5*	
4	14		15.1	
5	14		11.9	.97
6	14		13.7	
		Mean	*13.6*	
		3. Pastry mix		
1	50	top	0.3	0.006
2	52	top	.2	.0038
3	107	top	.3	.00288
4	108	top	.7	.0065
		bottom	.4	.0037[b]
		Mean p.p.m./day		*0.00479*
		4. Flour		
1	109	1-cm. layer	14.2	0.13
		2-cm. layer	.4	.00367
		center	.0	.0
2	110	1-cm. layer	17.5	.159
		1-cm. layer	17.5	.159
		5. Soup Mix		
1	133		0.0	0.0
2	141		4.7	.0334
3	141		.0	.0
4	141		.4	.00284
		Mean p.p.m./day		*.00906*
		6. Shredded coconut		
1	131	top	1.2	0.00915[c]
		bottom	5.3	.0405[c]
2	131	mixed	2.7	.0206
3	131	mixed	4.4	.0338
		Mean p.p.m./day		*.03468*

[a] Approximate air concentration during test = 0.038 micrograms per liter.
[b] Not included in calculation of mean.
[c] Samples from same package

packaged shredded wheat is a characteristic adsorption phenomenon; an initial high rate of sorption followed by a very gradual rate of sorption. Evidently the unpackaged shredded wheat had not reached an equilibrium state after two weeks' exposure since the concentration of lindane was below that of the packaged shredded wheat which had been exposed for a longer period of time. The high concentration of lindane found on the shredded wheat is indicative of the very large surface area offered by this substance.

The pastry mix packages again exhibited the stratification phenomena observed in the shredded wheat although the total concentrations are considerably less than those of the shredded wheat samples. The bottom flap constitutes 7.5% of the total area of the bottom half of the package. If the difference in concentrations is due to the bottom of the package being covered, the concentration at the bottom half should be about 92.5% of the concentration at the top half. However, the concentration in the half of the package was 0.04 p.p.m. or 5.7% of the top concentration (0.7 p.p.m.). Evidently some other factor is involved; perhaps a disruption of the air flow over the package (this package was well sealed).

The packages of flour exhibited a high concentration of lindane at the flour surface adjacent to the sides of the package. The concentration rapidly decreased toward the center of the package. This is to be expected since flour is said by Shepard (1951) to be very sorptive. The high sorption rate at the outer limits of the mass would tend to decrease the rate of vapor diffusion toward the center. Another factor that may account for this phenomenon is that flour is a fine particulate material, capable of being packed quite firmly. The spaces between particles are reduced considerable affording little space for the vapor molecules to penetrate into the mass.

Although aluminum foil is reputed by Queen (1953) to be almost impermeable to lindane vapor, variations in lindane concentrations occurred in the aluminum foil packages sampled. Two samples of soup mix had no detectable lindane while another sample had a slight amount and the fourth sample had a significant quantity. These variations in penetration may be due to "pin" holes in the aluminum foil. It is understood that such defects do occur in some grades of aluminum foil.

The shredded coconut package again exhibited a stratification similar to the shredded wheat and the pastry mix samples. However, the concentrations were reversed; this factor could be explained when the seals at both ends of the package were examined. The bottom seal was much less effective than the top, the top being a crimped heat seal; the former was a simple fold cemented onto the package wall.

From the data (packaged samples) there is little evidence to indicate a variation in the sorption of lindane with time. The packaged shredded wheat and pastry mix show a nearly uniform rate of contamination. On the other hand, the unpackaged shredded wheat appears to exhibit a typical adsorption curve, an initial rapid rate followed by a lowered rate of adsorption. The data suggests that the rate of contamination of packaged foods is limited by the package pentration rate of the insecticide.

Table 2.—Lindane vapor penetration of packaging materials.

Packaging Material	Total Exposed Surface Area[a] (Sq. Cm.)	Mean P.p.m. per Day	Mean P.p.m. per Day per Square Meter	Effectiveness of Package Closure
Boxboard (0.020 inch)	785	0.129	0.164	Top ineffective
Kraft paper	653	.149[b]	.228	Effective
Two laters of cellophane	225	.03468	.154	Bottom ineffective
Aluminum laminated to paper	206[c]	.00906	.0435	Effective
Outer box, box-board (0.020 inch)	306	.00325	.0106	Effective
Inner bag, 47-pound laminated liner paper				
Outer box, boxboard (0.0175 inch)	1,259	.00124[d]	.00099[d]	Effective
Inner bag, 25-pound glassine				

[a] Bottom surface of package disregarded.
[b] One centimeter layer.
[c] Disregarded outer boxboard package.
[d] Top.

The long periods of exposure described in this paper are in contrast to the quick turn-over of stock normally occurring in large supermarkets from which the original stock was purchased. These shorter periods of exposure would result in a lower uptake of lindane vapor. In food-storage areas, foods are generally held in their packing cases which commonly consist of three-layered corrugated protective cartons. This would presumably result in a considerably lower uptake of lindane vapor.

A comparison of protection afforded by various packages against lindane vapor is given in table 2.

An infestation of German cockroaches was controlled during the test period. House fly control was also quite effective.

SUMMARY AND CONCLUSIONS.—A series of packaged foods were exposed to continuously vaporized lindane at the rate of approximately 1 gram per 16,240 cubic feet per 24 hours. The foods were exposed for varying periods of time up to 4 months, then were analysed for lindane.

Transmission of lindane vapor into food packages occurred predominantly in the upper half of the package when the package was resting on a horizontal surface (Table 1). No commercial package (boxboard, kraft paper, cellophane, glassine or aluminum foil) was found to resist the penetration of lindane vapor completely (Table 2). The effectiveness of package closures markedly affected the penetration of lindane vapor. The order of decreasing penetration of packaging materials was boxboard, kraft paper, cellophane (two layers), aluminum foil laminated to paper, boxboard with a laminated liner paper, and boxboard with a glassine liner. Although no packaging material prevented penetration, some materials markedly reduced penetration.

REFERENCES CITED

Essig, E. O., W. M. Hoskins, E. G. Linsley, A. E. Michelbacher, and R. F. Smith. 1943. A report on the penetration of packaging materials by insects. Jour. Econ. Ent. **36**(6): 822–9.

Gerhardt, P. D. and D. L. Lindgren. 1954. Penetration of various packaging films by common stored-product insects. Jour. Econ. Ent. **47**(2): 282–7.

Hornstein, I., and W. N. Sullivan. 1953. Determination of lindane in air. Anal. Chem. **25**(3): 496–8.

Queen, W. A. 1953. Distribution and adsorption characteristics of vaporized lindane. Ass'n. Food & Drug Officials of U. S. **17**(4): 127–39.

Schechter, M. S. and I. Hornstein. 1952. Colorimetric determination of benzene hexachloride. Anal. Chem. **24**(3): 544–8.

Shepard, H. H. 1951. The chemistry and action of insecticides. p. 383.

The Inhibition of Cholinesterase and Succinoxidase by Malathion and Its Isomer[1]

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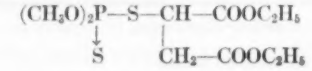
Malathion has the formula:



It was introduced in 1950 and is of particular interest as one of the first organophosphorus insecticides with low mammalian toxicity, a property which has led to its widespread use today. The *in vitro* effectiveness of the compound has only been studied against cholinesterase (Dubois *et al.* 1953, Metcalf & March 1953a, 1953b) and it was found to be a poor inhibitor of the enzyme, although its effectiveness increased on isomerization (Metcalf & March 1953a). As a succinate derivative it was possible that it might interfere with carbohydrate metabolism. This has been confirmed in the present work for the succinoxidase systems of mouse liver and house fly.

METHODS.—Except where otherwise stated, the cholinesterase used was that of diluted human plasma, and its activity was determined by a standard Warburg procedure (Spencer & O'Brien 1953) at 25° C. The insect nerve cholinesterase determinations were made upon preparations homogenized in water in a Potter-Elvehjem homogenizer immersed in ice and water; in this case the substrate was acetyl choline bromide (Eastman Kodak) at a final concentration of 1.2×10^{-2}M. Erythrocyte cholinesterase was prepared from human blood by the method of Adams (1949), and assayed under the same substrate conditions as the insect enzyme. Succinoxidase determinations were made on frozen liver of rat or mouse homogenized as above in .067 M phosphate buffer pH 7.2 to give 125 mg. of tissue per ml. Two ml. of this preparation were used, with 0.2 ml. of 0.4 M sodium succinate (Eastman Kodak) as substrate. Fly succinoxidase determinations were similar, but used a 10% homogenate of 2- or 3-days-old *Musca domestica.* The standard Warburg procedure was used, with an oxygen atmosphere and at 25° C.

Insect injections were made with an Agla micrometer syringe into the abdomen, slightly laterally. Solutions in propylene glycol were used. The cockroaches used were *Periplaneta americana* (L.), the flies were *Musca domestica.*

Malathion of 99% purity was used; the determination of phosphorus in it and its products required a vigorous hydrolysis to orthophosphate, for which the molybdate-catalyzed method of Simmons & Robertson (1950) proved satisfactory when adapted for semi-micro quantities, giving 99% recoveries. Orthophosphate was measured by the method of Rockstein & Herron (1951).

RESULTS.—*Isomerization.*—Metcalf & March (1953a) have shown that malathion isomerizes on heating. Further information on the structure and properties of the isomer was sought. Observations were made on the influence of time and temperature of incubation upon the isomerization of malathion, using undiluted samples sealed in glass vials (air atmosphere). Depending upon the severity of conditions, products varying from light brown (115° C., 18 hours) to charred black (180° C., 4 hours) are obtained. Except under very mild or very extreme conditions, two fractions are seen, a brown supernatant liquid insoluble in water but readily soluble in organic solvents, and a whitish glassy solid phase which is very hard, completely insoluble in all the organic solvents used, but which dissolves with crackling and heat upon addition of water. If only a little water is used, a heavy white floc is seen, but this dissolves if more water is added.

The approximate distribution of material in these fractions was studied, using phosphorus as index. Recoveries were poor because of the intractable nature of the solid phase under anhydrous conditions, being difficult to crush and extract with the solvent to recover all of the solvent-soluble phase. At 115° C. for 18 hours, 75% was found in the liquid phase; at 150° C. for 4 hours 25% was in the liquid and 66% in the solid phase; at 180° C. for 4 hours 6% was in the liquid and 85% in the solid phase.

Aqueous solutions of the solid phase inhibited cholinesterase poorly; pI_{50}'s[2] between 0.8 and 2.0 were obtained with different samples, the variability again arising from difficulty in washing the material. This activity is of the same order as that of malathion, which is however not soluble in water. Chloroform solutions of the liquid phase were evaporated to dryness and taken up in 50% ethanol (at lower concentration, e.g., for 0.1% solution, 5% ethanol could be used) and the effectiveness against cholinesterase determined using appropriate ethanol levels in the controls. The results (given in detail below) showed that this phase contained an inhibitor very much more potent than the parent material. In the work de-

[1] Contribution No. 48. Accepted for publication January 18, 1956.

[2] The following terminology is used in this paper: pI: negative logarithm of molar concentration. pI_{50}: pI that gives 50% enzymic inhibition. LD_{50}: Dose that is lethal to 50% of a population.

scribed below the term "isomalathion" is reserved for this component.

The isomalathion used in the work reported below was prepared by heating malathion in a sealed vial at 150° C. for 3 hours, taking up as much as possible in chloroform and storing in chloroform solution. When required the chloroform was removed under vacuum and the residue taken up in ethanol.

In figure 1 the infrared spectra of malathion, isomalathion and the solid phase from isomerization are recorded. To obtain a spectrum of the last, a film of malathion was introduced between two rock salt plates, which were then sealed in a tube and held at 150° C. for 3 hours. The plates were then separated, washed with chloroform and used in the spectrophotometer. In the malathion spectrum, the 655 $cm.^{-1}$ peak is probably attributable to P→S (Daasch & Smith 1951) and recent work by Henglein *et al.* (1954) suggests that the same group gives the 820 peak. Both these peaks are strongly reduced on isomerization. There is a corresponding increase in the peak at 1,295 which is almost certainly due to P→O (Holmstedt & Larsson 1951, Williams 1951). A decrease is noted in the 1020 peak which is attributable to P−O −C. New peaks were produced at 1,645 and 980 by isomerization.

These data all support the hypothesis that the isomerization consists of an interchange between a sulphur of the P→S and an oxygen of a methoxy group. This is similar to the isomerization paths suggested by Martin (1949) for parathion and by Metcalf & March (1953a) for its methyl analogue.

The infrared spectrum of the solid phase is diffuse but shows broad peaks in the hydroxyl (3,400), the carbonyl (1,600 to 1,800) and phosphoryl (1,265) regions. Probably there is a mixture of breakdown products, and the spectrum and the behaviour with water suggests the presence of anhydrous phosphoric acids.

The ultraviolet spectra of malathion and its isomer

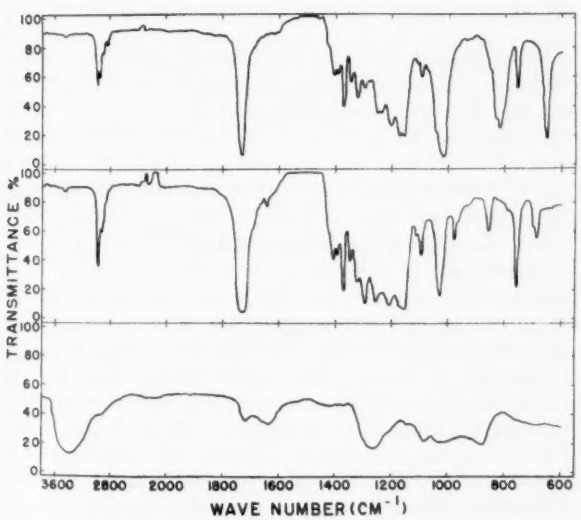


FIG. 1.—Infrared spectra of malathion (top), isomalathion (centre) and the solid phase from heat-treatment of malathion (bottom).

were measured, using 0.02% solutions in ethanol. Each gave a smooth sigmoid curve with no peaks or shoulders, but with malathion the approximate spread of the sigmoid was 220 to 280 mμ, for isomalathion it was 220 to 400 mμ, so that on this basis the proportion of the two components in a mixture could be determined by measuring transmittance at, say, 300 and 240 mμ.

In order to obtain an estimate of the stability of isomalathion, the rate of loss of anticholinesterase activity was studied. The material was dissolved in 50% ethanol at 25° C. A half-life of about 45 hours was found.

Observations IN VIVO.—The effects of malathion poisoning were studied in order to provide clues as to the mode of action. The LD_{50} of malathion against adult American cockroaches was 8.4 γ/g. The isomer was much less toxic, with the LD_{50} of the order of 60γ/g. The non-toxicity of malathion to mammals has been well established by Johnson *et al.* (1952), Dubois *et al.* (1953) and Plao't (1953). Isomalathion at 50γ/g. gave no symptoms following intravenous injection into mice.

Malathion poisoning in cockroaches gives, at first, the usual organophosphate effects: some hyperexcitability followed by complete ataxia with marked tremors, initially involving the whole body, but later primarily the limbs. However the onset of the ataxic phase is moderately rapid (½ to 1 hour) yet extraordinarily prolonged, so that at doses of 10 γ/g. some of the cockroaches were completely moribund 5 days after injection, yet showed intermittent tremors, especially upon stimulation. Even at doses of 100 γ/g. a similar condition persisted for 4 days after injection. None of these severely affected cockroaches recovered, so that in counts of LD_{50}'s it was safe to include them among the mortalities. In contrast, death occurred in cockroaches poisoned with TEPP within 5 to 10 minutes of injection, and the hyperexcitable symptoms were correspondingly stronger, involving vigorous flying movements and an arching of the whole body. Chamberlain & Hoskins (1951) studying a series of 18 organophosphates injected into cockroaches gave average times to death that in no case exceeded 50 hours, and in those cases where the time was over 26 hours, symptoms did not develop for at least 1 hour, and usually not until 5 or more hours after injection.

The respiration of malathion-poisoned insects was next investigated. The method was similar to that of Harvey & Brown (1951) but injections were made in propylene glycol and *Periplaneta* nymphs were used in place of *Blattella* adults. Four untreated insects, four propylene glycol treated, and eight malathion treated insects were studied. Doses of from 17 to 96 γ/g. were used. There was no consistent difference in the respiration patterns, examples of which are given in figure 2, and certainly no sign of the initial marked enhancement of respiration which Harvey and Brown noted with the organophosphates they studied. In some poisoned insects the curves showed quite marked peaks and troughs during the first 350 minutes.

The cholinesterase levels in the nerve cords of poisoned cockroaches were studied. Examination of all the data obtained in these experiments showed that there was no correlation between cord weight and cholinesterase activity, in agreement with the findings of Chadwick &

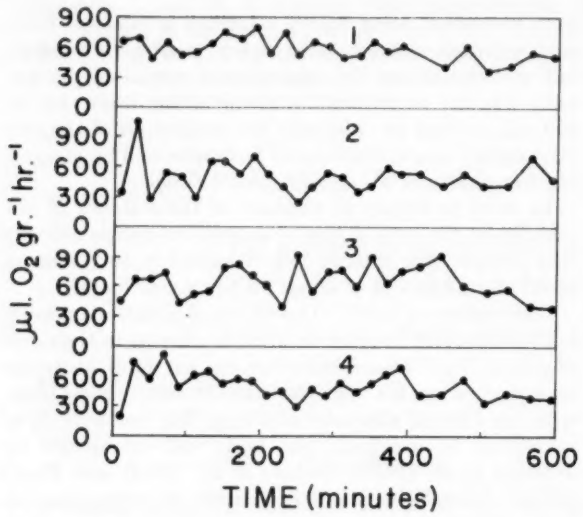


FIG. 2.—Effect of malathion treatment on the respiration of cockroach nymphs. 1, 2, Controls (injected with propylene glycol). 3, malathion 27 γ/g. 4, malathion 42 γ/g.

Hill (1947). The activities of the cords were therefore used uncorrected for their weights.

Table 1 shows the cholinesterase levels of cockroaches 5 days after injection with malathion. This long period was used because of the considerable mortality time (see section on toxicity). In no case did the inhibition exceed 48%. Table 2 shows the results of acute experiments, and comparative results for TEPP used as a "classical" anticholinesterase. There is a much greater inhibition of cholinesterase than in the long term experiment of table 1. This recovery, after an initial high inhibition, was confirmed by studying the cholinesterase levels of poisoned cockroaches at various times after poisoning. Cords were removed and stored in a mixture of equal volumes of glycerol and 0.9% saline at −18° C., then all assayed on the final day. The results are shown in table 3. All the above results threw doubt on the importance of cholinesterase in poisoning by malathion. More extensive studies, and on other enzyme systems, were suggested.

An experiment was carried out using 2-day-old house flies divided into 8 batches of 60 flies each (30 of each sex). The batches were paired and the flies of one batch of each pair were treated topically with 1 µl. of 0.04% malathion in acetone, giving a dose of 27 γ/g. Each pair of batches was then placed in the freezer at a different time-interval following treatment. When all had been frozen they were removed and each batch was homogenized in 10 ml. of 0.9% KCl and assayed for cholinesterase and succinoxidase, with the results shown in figure 3. It is evident that cholinesterase, after a strong initial inhibition (98%) recovers rapidly and by the time the insects start to die, inhibition is only about 55%. Succinoxidase is only slightly inhibited (16% initially).

In order to interpret these results, the effect of death on these enzymes had to be known; if the level of either varies much upon death (and especially when death is followed by exposure at room temperature), the variations in level might be caused by death rather than be its cause. One batch of a pair was killed by exposure to carbon tetrachloride vapour and left at room temperature 24 hours, the other batch was frozen at zero time. The exposed flies had succinoxidase levels of 9 µl. O_2 hr.$^{-1}$, the frozen flies 80 µl. O_2 hr. $^{-1}$. The corresponding cholinesterase levels were 388 and 406 µl. CO_2 hr.$^{-1}$. Another pair of batches which had been stored in the deep-freeze for several days was used in the next experiment. One batch was exposed at room temperature for 3 hours, then replaced in the freezer. Both batches were later assayed; the exposed flies had a succinoxidase level of 66 µl. O_2 hr.$^{-1}$, the unexposed 90. The corresponding cholinesterase levels were 187 and 192 µl. CO_2 hr.$^{-1}$.

Christie & Judah (1954) have shown that carbon tetrachloride can effect mitochondria, and freezing and thawing doubtless have a disruptive effect; it was therefore felt to be desirable to kill the flies with a poison whose mode of action was probably unrelated to mitochondrial or nerve effects (for the cholinesterase studies). Chlorine was used, and momentary exposure of the flies to a trace of the gas was immediately lethal. The flies were then divided into several batches of 30 each, and at various times batches were placed in the deep-freeze.

Table 1.—Cholinesterase inhibition in cockroach nerve cord 5 days after malathion injections.

Dose (γ/g.)	Condition After 5 Days	b_{30} per Cord[a]	Per Cent Inhibition
0	alive	27	0
4.3	alive	21	22
8.5	alive	20	26
43	alive	15	44
43	dead	14	48
85	alive	17	37
85	dead	25	7

[a] b_{30} = µl of CO_2 produced in 30 minutes in cholinesterase assay.

Table 2.—Cholinesterase inhibition in cockroach cords 5 hours after injecting tetraethylpyrophosphate or malathion.

Compound	Dose (γ/g.)	b_{30} per Cord	Per Cent Inhibition
—	0	36	—
TEPP	10	1.9	96
TEPP	1	2.9	20
Malathion	85	5.9	84
Malathion	9	15.0	58

Table 3.—Cholinesterase inhibition in cockroach cords following injection of 50 γ/g. of malathion.

Time After Injection	b_{30} Per Cord	Per Cent Inhibition
2 hours	14	61
1 day	28	22
2 days	29	19
3 days	29	19
4 days	28	22
No injection	36	—

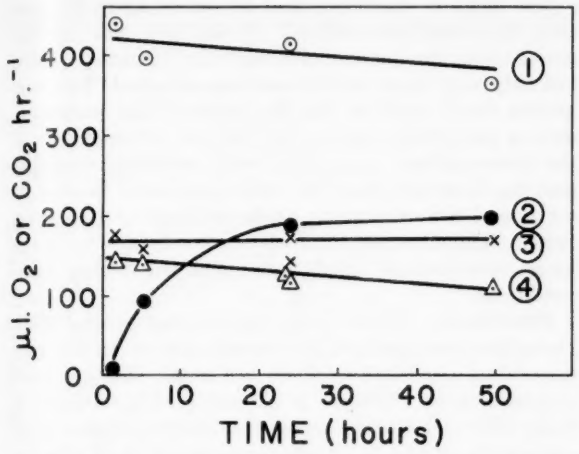


Fig. 3.—Effect of malathion treatment (at zero time) on cholinesterase and succinoxidase of house fly. 1. Cholinesterase-untreated flies. 2. Cholinesterase-treated flies. 3. Succinoxidase-untreated flies. 4. Succinoxidase-treated flies. At 2 hours, 50% of the treated flies were tremoring; at 50 hours 50% were dead, at all times 50% appeared normal.

When all were frozen, they were removed and each batch ground in 5 ml. of 9% KCl, then assayed as before. The results given in figure 4 show that the succinoxidase level falls with time, being after 3 days only 45% of the 1.5 hour level. Over the same period the cholinesterase level remains approximately constant.

The effect of death upon the levels of succinoxidase and cholinesterase thus follows the same pattern with all three methods of killing. It was also observed that whenever dead flies had been allowed to stand at room temperature for about 1 day or for a few hours after deep-freezing the homogenate from them was yellow instead of the usual red. The yellowness was roughly paralleled by the decrease in succinoxidase.

Observations in vitro.—Studies *in vitro* of the two enzymes were carried out in order to obtain further information about their susceptibility to malathion and to explain the selective toxicity of the compound. The time course of cholinesterase inhibition by malathion was followed, as a guide to appropriate incubation periods and in order to investigate the kinetics of inhibition. The results for serum cholinesterase are given in figure 5 plotted as described by Aldridge (1952). The non-linearity of the plot indicates that the reaction between malathion and cholinesterase does not follow simple first order kinetics. This could be due to traces of an unstable inhibitory impurity, or to destruction of the malathion. Now that Mounter (1954) has shown that TEPP, di-isopropyl fluorophosphate, ethyl p-nitrophenyl thiobenzenephosphonate, and para-oxon are all hydrolyzed by serum, the latter possibility existed. Experiments were carried out in the Warburg apparatus at 25° C. using 1 ml. of whole human plasma or 10% homogenate of house fly or cockroach, with a bicarbonate buffer, tipping in 0.2 ml. of ethanol solutions of malathion and isomalathion to give a final concentration 1.5×10^{-2} M. No extra carbon dioxide was evolved, evidence for the absence of a malathion-hydrolyzing enzyme. The results in figure 5 suggest that the observation made by Metcalf & March (1953b) in a footnote, that a certain concentration of malathion giving 91% inhibition of fly cholinesterase after 1 hour incubation gave only 34% inhibition when the incubation period was omitted, is explained by a time effect alone, without implying activation by the fly brei. Ninety minutes was selected as a suitable time of incubation in later cholinesterase studies.

The inhibition by isomerized malathion was also followed (Figure 5) and conformed to the kinetics of a first

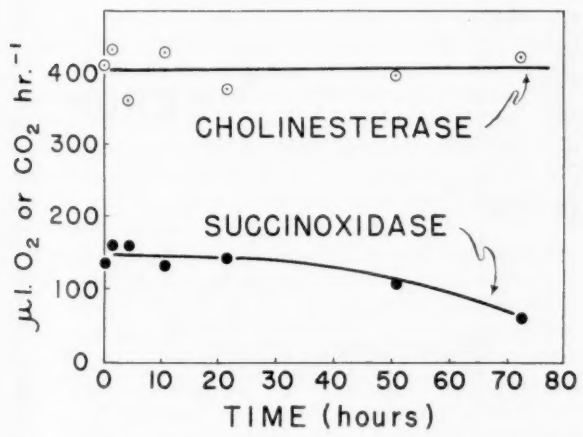


Fig. 4.—Effect of standing at room temperature upon cholinesterase and succinoxidase in chlorine-killed house flies.

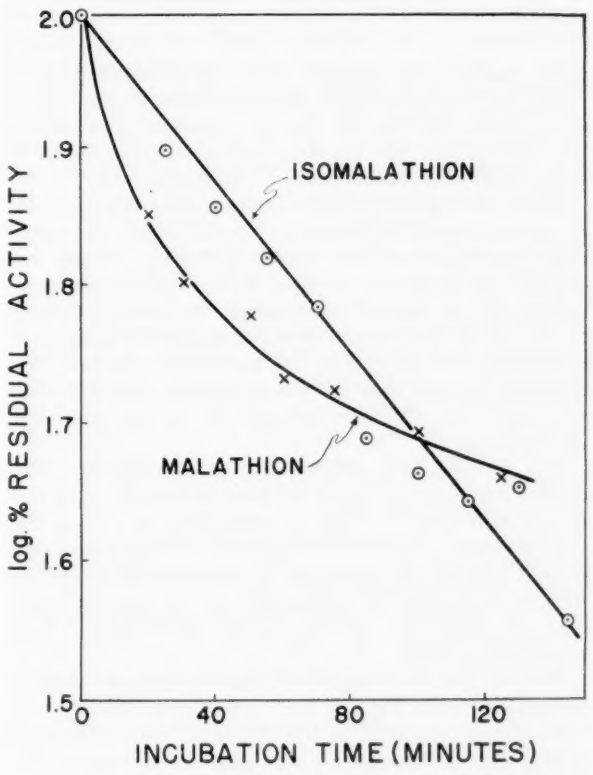


Fig. 5.—Effect of time of incubation on cholinesterase inhibition by malathion and isomalathion. For details see text.

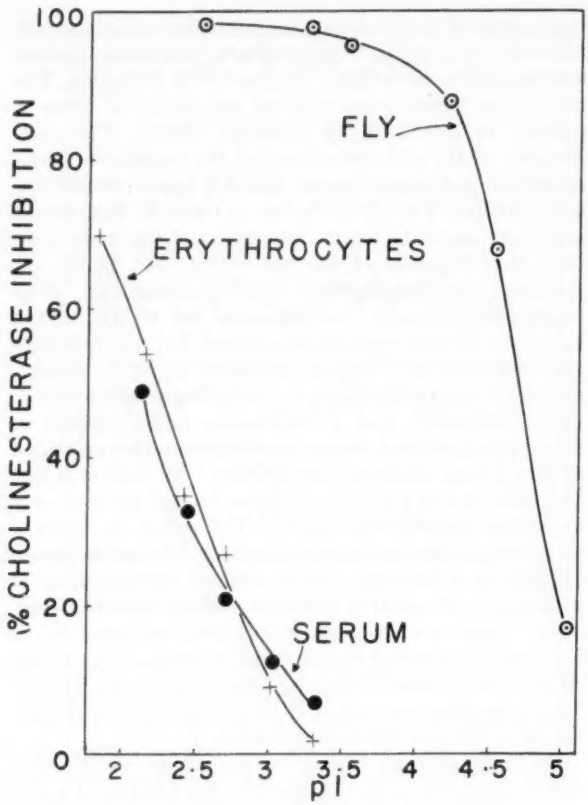


FIG. 6.—Effect of malathion on various cholinesterases.

order reaction. By analogy with the work of Aldrige (1952) on various other organophosphates this is a case of a bimolecular reaction with one component in excess. The bimolecular rate constant calculated from figure 5 is 3.78×10^2 litre moles^{-1} min.$^{-1}$ for isomalathion.

The inhibitions of the cholinesterase of fly head, human serum and human erythrocytes by malathion and isomerized malathion are shown in figures 6 and 7. It was observed that unaltered inhibitor is 300 times as effective against fly as against mammalian cholinesterases and the isomer is 100 times as effective against fly as against erythrocyte cholinesterase. The isomer is a substantially stronger inhibitor than the parent product for all cholinesterases; over 300 times stronger in the case of serum cholinesterase.

The succinoxidase effect was next investigated. The results are given in figure 8. Both malathion and the isomer were found to inhibit mouse liver and house fly succinoxidase, the isomer being over 10 times more effective. The abrupt flattening of the malathion curve for liver succinoxidase is attributable to the limited solubility of malathion; at these levels the solutions were excessively turbid.

Since it has been suggested that even such organophosphates as TEPP and DFP produce an effect upon carbohydrate metabolism (Brooks *et al.* 1949, Michaelis *et al.* 1949) an experiment was made to determine the effect of TEPP on rat liver succinoxidase, but no inhibition was found even at final concentrations of 10^{-2} M.

The effect of malathion and its isomer upon the succinic dehydrogenase activity of rat liver was investigated using the method of Quastel & Wheatley (1938) but only very small inhibitions were obtained. This surprising result could be due to the fact that malathion acts on the cytochrome oxidase system. Alternatively, if the succinoxidase preparation were oxidizing succinate past the fumarate stage, the malathion could be inhibiting one of the subsequent oxidative steps of its carrier system. This does not seem very probable in view of the large succinoxidase inhibitions obtained under some conditions.

DISCUSSION.—It has been shown that several thiophosphates are oxidized by various tissues to the corresponding phosphates, which are much stronger anticholinesterases (Metcalf & March 1953b, Diggle & Gage 1951). Isomerization of the thiophosphates gives compounds similar in anti-enzyme activity and stability to the oxidation products. One might expect malathion to be oxidized to the hypothetical "malaxon" (O,O-diethyl S-(1,2 dicarbethoxyethyl) phosphorothiolate), as the work of Metcalf & March (1953b) suggests. Until

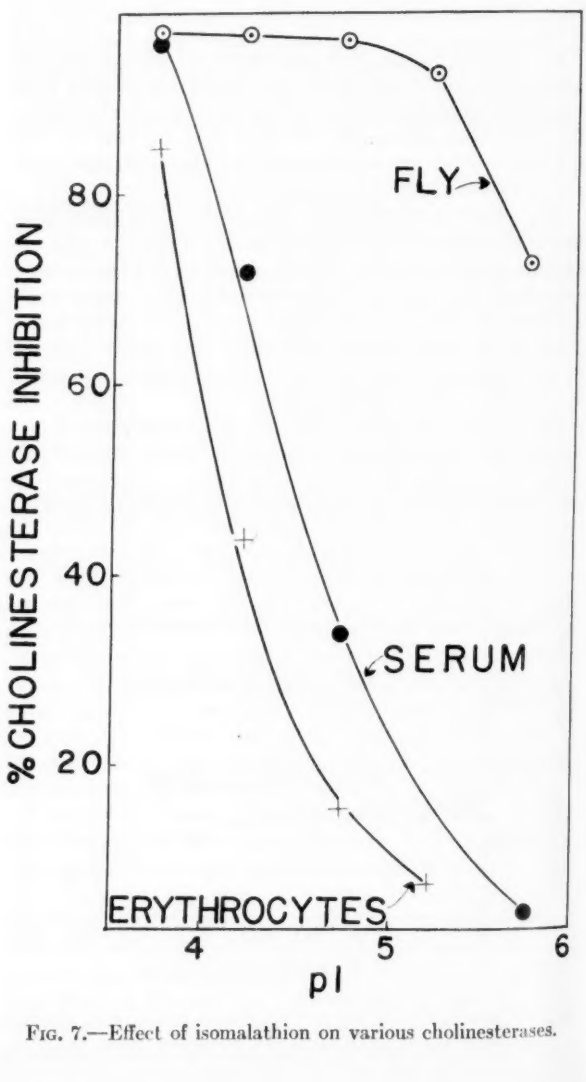


FIG. 7.—Effect of isomalathion on various cholinesterases.

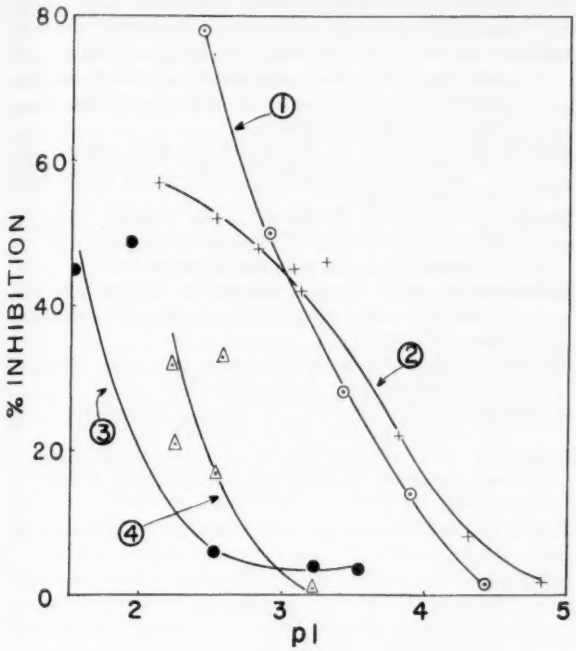


Fig. 8.—Effect of malathion and isomalathion on various succinoxidases. 1. Isomalathion against liver. 2. Isomalathion against house fly. 3. Malathion against liver. 4. Malathion against house fly.

"malaoxon" is available, its properties may be taken to be approximated by the isomer which has been used in the present study.

Three criteria of a hypothesis of mode of action are (a) that the system which is suggested as being primarily affected can be inhibited by low concentrations of the poison (b) that the system is of importance to the organism; (c) that there is a correlation between the symptomatology of poisoning and the function of the system.

Malathion treatment of insects gives symptoms typical of nerve poisoning. The commonest cause of such effects is anticholinesterase action, but Peters (1953) has shown in several cases that "biochemical lesions" in carbohydrate metabolism lead to convulsions and similar nervous symptoms. The same work shows the lethal nature of agents which can produce such lesions. The problem therefore reduces to considerations of the sensitivity of the systems under study, and since cholinesterase and succinoxidase (selected as one of many possible important enzymes) are present together in the nerve, *in vitro* results should give valuable clues.

The patterns of activity, respiration and cholinesterase levels following poisoning by typical anticholinesterases (Chadwick & Hill 1947, Chamberlain & Hoskins 1951, Harvey & Brown 1951) differ from those of malathion; in malathion poisoning the pre-lethal period is extraordinarily prolonged, there is no stimulus of respiration and nerve cord cholinesterase is only temporarily inhibited to any marked extent. It is especially interesting that the first deaths occurred when the cholinesterase inhibition was only about 57%. The work of Nachmansohn (1948) on conduction in nerves suggests that cholinesterase is commonly present in an excess of 10 times, and the cholinesterase levels given by Chamberlain & Hoskins (1951) for cockroaches poisoned by organophosphates suggest a similar excess. The fact that death coincides with relatively high cholinesterase levels and not with the period of extreme inhibition is strong evidence against a causal relationship. However, it is total cholinesterase that is being studied, and it is not impossible that a localized and important site of the enzyme is following a different pattern of inhibition and recovery.

The succinoxidase results of figure 3 are shown by the experiments on the effect of exposure to be unreliable, once deaths have begun to occur. Certainly the initial inhibitions are small (e.g. 16% at 1.75 hours) and there is little evidence for linking them with death. Unfortunately there is no information available as to the extent of inhibition the oxidative enzymes can undergo without resulting in death. Furthermore, if malathion inhibition of succinoxidase is reversible, the large dilution of inhibitor which follows homogenization of the tissues would give rise to low estimates of the inhibition *in vivo*.

The pI_{50} against succinoxidase is about 2.0 for malathion and 3.0 for the isomer. The fact that there is little difference in sensitivity between mouse and fly succinoxidase is evidence against the significance of inhibition of this system.

Malathion had pI_{50}'s of about 2.1 and 4.7 against mammalian and fly cholinesterase respectively; the isomer had corresponding pI_{50}'s of 4.5 and 6.0. The values are quite compatible with the finding that mammals are resistant to malathion poisoning and with the suggestion that in insects the effective anti-enzyme is a conversion product of malathion.

The case for cholinesterase inhibition as the cause of death is thus supported by *in vitro* findings but the *in vivo* evidence is against it. The findings with regard to succinoxidase inhibition do not favour the implication of this enzyme. Further study of the effect of malathion upon other metabolic systems is indicated.

Summary.—Infrared studies on the effect of heat on malathion indicate that isomerization to the S-methyl thiolphosphate (isomalathion) occurs. Following malathion treatment of cockroaches, symptoms unlike those typical of organo-phosphate poisoning were observed. Cholinesterase showed a preliminary sharp depression and then rose until death; succinoxidase was slightly inhibited. Regardless of the method of killing (freezing, carbon tetrachloride or chlorine) succinoxidase levels dropped if flies were exposed at room temperature after death, but cholinesterase levels remained constant over the 3-day period studied. Isomalathion was *in vitro* a stronger inhibitor than malathion of human serum and erythrocyte cholinesterase, fly cholinesterase, mouse liver succinoxidase and fly succinoxidase. The *in vitro* findings support the suggestion that malathion kills by an anticholinesterase action, but there are anomalies in the *in vivo* results. Death in the cockroach is probably not attributable to succinoxidase inhibition.

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J. H. Walton are gratefully acknowledged. The malathion was donated by the American Cyanamid Company.

REFERENCES CITED

Adams, D. H. 1949. The specificity of the human erythrocyte cholinesterase. Biochim. et Biophys. Acta. **3**: 1–14.

Aldridge, W. N. 1952. Some properties of specific cholinesterase with particular reference to the mechanism of inhibition by E605 and analogues. Biochem. J. **46**: 451–60.

Brooks, V. B., R. E. Ransmeier, and R. W. Gerard, 1949. The action of anticholinesterases, drugs and intermediates on respiration and electrical activity of the isolated frog brain. Amer. J. Physiol. **157**: 299–316.

Chadwick, L. E., and D. L. Hill. 1947. Inhibition of cholinesterase by di-isopropyl fluorophosphate, physostigmine and hexaethyl tetraphosphate in the roach. J. Neurophysiol. **10**: 235–46.

Chamberlain, W. F., and W. M. Hoskins. 1951. The inhibition of cholinesterase in the American roach by organic insecticides and related phosphorus-containing compounds. Jour. Econ. Ent. **44**: 177–91.

Christie, G. S., and J. D. Judah. 1954. Mechanisms of action of carbon tetrachloride on liver cells. Proc. Roy. Soc. (B) **142**: 241–57.

Daasch, L. W., and D. C. Smith. 1951. Infra-red spectra of phosphorus compounds. Anal. Chem. **23**: 853–68.

Diggle, W. M., and J. C. Gage, 1951. Cholinesterase inhibition by parathion *in vivo*. Nature. **168**: 998.

Dubois, K. P., J. Doull, J. Deroin, and O. K. Cummings. 1953. Studies on the toxicity and mechanism of action of some new insecticidal thionophosphates. A.M.A. Arch. Indust. Hyg. **8**: 350–8.

Harvey, G. T., and A. W. A. Brown, 1951. The effect of insecticides on the rate of oxygen consumption in *Blatella*. Canad. J. Zool. **29**: 42–53.

Henglein, A., G. Schrader, and R. Muhlmann. 1954. Quantitative infrarotspektroskopische Bestimmung des Isomerenverhaltnisses in dem System-Insecticid "Systox." Zeit. f. anal. Chem. **141**: 276–81.

Holmstedt, B., and L. Larsson, 1951. Infra-red spectra of some organic phosphoryl compounds. Acta. Chim. Scand. **5**: 1179–86.

Johnson, G. A., J. H. Fletcher, G. K. Nolan, and J. T. Cassaday. 1952. Decreased toxicity and cholinesterase inhibition in a new series of dithiophosphates. Jour. Econ. Ent. **45**: 279–83.

Metcalf, R. L., and R. B. March, 1953a. The isomerization of organic thionophosphate insecticides. Jour. Econ. Ent. **46**: 288–94.

Metcalf, R. L., and R. B. March, 1953b. Further studies on the mode of action of organic thionophosphate insecticides. Ann. Ent. Soc. Amer. **46**: 63–74.

Michaelis, M., N. I. Arango, and R. W. Gerard. 1949. Inhibition of brain dehydrogenases by anticholinesterases. **157**: 463–7.

Mounter, L. A. 1954. Some studies on enzymatic effects of rabbit serum. Jour. Biol. Chem. **209**: 813–7.

Nachmansohn, D. 1948. The role of acetylcholine in conduction. John Hopkins Hosp. Bull. **83**: 463–93.

Peters, R. A. 1953. Significance of biochemical lesions in the pyruvate oxidase system. Brit. Med. Bull. **9**: 116–22.

Plao't, N. 1953. Malathion, a new insecticide. Hassadeh **34**: 54–55.

Quastel, J. H., and A. H. M. Wheatley. 1938. Anaerobic oxidations: on ferricyanide as a reagent for the manometric investigation of dehydrogenase systems. Biochem. Jour. **32**: 936–43.

Rockstein, M., and P. W. Herron. 1951. Colorimetric determination of inorganic phosphorus in microgram quantities. Anal. Chem. **23**: 1500–1.

Martin, H. 1949. Organophosphorus insecticides. Manuf. Chem. **20**: 158–65.

Simmons, W. R., and J. H. Robertson, 1950. Determination of total phosphorus in organic phosphates. Anal. Chem. **22**: 294–6.

Spencer, E. Y., and R. D. O'Brien. 1953. Enhancement of anticholinesterase activity in octamethyl pyrophosphoramide by chlorine. Jour. Agr. Food Chem. **1**: 716–20.

Williams, E. F. 1951. Properties of *O,O*-diethyl *O*-*p*-nitrophenyl thiophosphate and *O,O*-diethyl *O*-*p*-nitrophenyl phosphate. Ind. Eng. Chem. **43**: 950–4.

The Khapra Beetle Problem in California[1]

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When the khapra beetle, *Trogoderma granarium* Everts, was first found in California in November 1953, its true economic significance as a pest of stored grain and seed was not apparent. It appeared to be just another species added to the eight species of *Trogoderma* already associated with stored grain in the state. In fact, it was this same reaction back in 1946 which resulted in no one submitting specimens for identification. These were recently confirmed from remaining cast skins as representing khapra beetle. Had they done so at the time, the present problem might have been materially simplified, and might have been restricted to this state. However, it is fortunate that it was finally identified before it became any more widely spread than at present indicated.

An immediate review of the meager literature available covering this species did show it to be a serious pest of barley, wheat, rice and peanuts in the old world. However, it failed to show its ever having been previously reported from the western hemisphere. There was not much information available as to its control or its life history and habits that might be helpful in evaluating the seriousness of the problem presented in its finding in California.

Any serious thought of attempting its eradication was at the time, dispelled by the results of early survey. At the same time steps were immediately taken and rigidly enforced restricting the movement of host seed from any known infested premise, to supervised approved origin treatment. Early state survey showed infestation to be scattered over several counties involving not only large

[1] Presented at the third Annual Meeting of the Entomological Society of America held at Cincinnati, Ohio, Nov. 28, 1955. Accepted for publication January 13, 1956.

warehouse storage and cereal processing plants, with extended channels of distribution, but included many private premises on which small lots of grain were held for on-farm use, either for planting or for feeding. From that standpoint these latter type of infested premises did not seem to present any particular hazard in immediately contributing to further outward spread, excepting as used bags might find their way back into channels of trade. This was fully safeguarded against by requiring and enforcing treatment as a condition of such movement. However, serious attention was immediately given to the possibility of reducing infestation in the larger distributing plants so as to at least delay further spread as long as possible. Owners of such premises were fully informed of the situation with the suggestion that they take every precaution against becoming infested or if infested, exercise as much care as possible so as not to be responsible for further spread.

SPRAYS INEFFECTIVE AGAINST WAREHOUSE INFESTATION.—By early spring of 1954 the original infestation at Alpaugh in Tulare County which involved 3700 tons of bulk barley in a simple, conventional, storage warehouse had progressed to a point where there were more larvae than seed in the surface 5 feet of grain. As rapidly as possible the house was emptied, each load being subjected to a heavy fumigation schedule using methyl bromide which assured complete mortality of all stages of the beetle present. When the house was completely emptied, the owner, in cooperation with state and federal entomologists, expended approximately $5,000 in applying control measures which, in addition to general sanitary cleanup, included repeated, drenching sprays of DDT, oil, malathion, parathion—in fact anything that showed any promise. Infestation was eventually reduced to the point where not a single living specimen could be found. Feeling perfectly safe, the building was then used to store the 1954 crop. By March of 1955 this new grain was more heavily infested than had been the previous storage. In other words, the spraying and sanitary measures had been of no permanent value in eliminating or even holding down infestation.

BEETLE'S HABITS SUGGESTED ERADICATION POSSIBLE.—In the meantime it had been learned that the beetle did not attack the growing crop and therefore field infestation was impossible. Also, that although the adult had wings it apparently did not use them in extending its range. Infestation was therefore restricted to individual premises offering an opportunity of complete suppression on that basis. The only obstacle to the success of such measures, if attempted, seemed to lie in the degree to which all existing infestation could be found.

FUMIGATION OF ENTIRE INFESTED STRUCTURE DEEMED NECESSARY.—There was one other important obstacle to the success of such a program, presented not so much by the lack of an effective control chemical but rather by the difficulty of accomplishing its effective application. Failure from the use of sprays apparently resulted from the inability to reach all of the larvae in the incredibly minute building crevices in which they found it possible to hide. Also to the fact, as later learned, that one type of larva had the ability, particularly in the presence of high populations, to hide out for long periods of time without food—as long as 3 or more years. It was obvious that nothing less than a penetrating fumigant could be expected to be effective under such conditions.

Another finding restricted even such an approach as fumigation to unusual measures. It was quickly learned that infestation was not limited to the interior of a building. Larvae could be found almost anywhere on the outside as well as within infested structures. Orthodox interior fumigation would therefore be ineffective no matter how tight the building might be. Also, there was no experience to serve as a guide in covering the large structures involved. Small buildings had previously been covered by contractors in the control of termites but nothing as large as the standard grain storage warehouse with its processing mills had evern been attempted. It was thought that even if possible, the cost would be prohibitive. Nevertheless, the State Department of Agriculture decided to determine just what could be done along that line.

TEST FUMIGATIONS.—Through the cooperation of the owner of an infested warehouse in the Imperial Valley in Southern California, which had been emptied of all grain storage, arrangements were made for its fumigation under gastight covers. The warehouse represented approximately a million cubic feet. Methyl bromide was selected as the fumigant with a thousand cubic feet being established as the unit of measurement in computing the dosage required. This unit was also used in establishing the cost of covering a structure with gastight tarpaulins.

A contractor was found who was willing to undertake the job at a little less than $4.00 per M cu. ft., in this case the total cost of covering was $3500. Subsequently this unit price on other jobs sky-rocketed to $18.00, with one structure of 3 million cubic feet costing $47,500 for cover alone. Under competitive bidding, however, and the accumulation by contractors of the large expanse of expensive plasticized nylon required, the cost has recently dropped back to between $5.00 and $6.00 per M cu. ft. which is proving reasonable and practical. It is admitted that the numerous buildings of varying size making up the average grain storage processing plant, with its maze of complicated towers, conveyors, catwalks and connecting pipes, plus the possibility of heavy wind damage from such a tremendous spread, does represent a real physical problem.

Methyl bromide was selected as the fumigant as it had been found by University of California workers to be biologically effective, under laboratory conditions, against all stages of the khapra beetle at concentrations of 3 to 4 pounds per M cu. ft. for 12 hours. It was known to be noninflamable, nonexplosive and less hazardous to warm-blooded animals than any other fumigant studied. At these concentrations it was later approved as meeting quarantine requirements.

However, in view of the high cost of covering buildings for fumigation, restricting such operations to a single treatment, it was arbitrarily decided to use 5 pounds per M cu. ft. for 48 hours, to assure complete mortality of all stages, under the most critical conditions that might be encountered.

Later, the federal authorities, who were primarily interested in protecting the other 45 noninfested states, agreed that if a concentration of 2 pounds of methyl bromide per M cu. ft. could be maintained at all sampling stations, representing the most critical points within the

structure, during any 24 hours of a continuous 48-hour exposure period, they would approve immediate release of the treated premise from any further quarantine restriction. Based on experience this formula has recently been modified to also accept 1½ pounds for 36 hours under similar conditions. This modification was made necessary by the difficulty and delay in obtaining and maintaining the higher concentrations in the presence of highly sorptive seeds such as milo, corn, flax, cottonseed and others. The guess-work was taken out of the situation by requiring sampling at all critical levels in the structure and its contents during fumigation using a thermal-conductivity direct analysis unit with readings made at regular intervals throughout the period of exposure.

One loses his sense of perspective in operations of this type—the unusual size of the structures involved running into millions of cubic feet; the cost of their covering with expensive gastight material amounting to $47,500 in one case to cover a 4-million-cubic-foot plant; the tremendous number of square yards of plasticized nylon used costing approximately $2.00 per square yard with 9½ acres required in covering one structure; the unusual amount and cost of the fumigant used—10 tons in the initial charge with an additional 2 tons to maintain concentrations in one instance—at a cost of $1,000 per ton. These are all entirely out of proportion to normal usage. They are cited merely to give some idea of the size of the problem presented to owners and regulatory officers in attempting eradication, in which upward of 200 premises are concerned.

RESULTS OF FUMIGATION TESTS.—In the first eradication test it was determined that the dosage and exposure used could be depended on to give 100% mortality of all stages of the khapra beetle in an empty infested grain storage warehouse.

A second test, involving bulk barley, using the same dosage of methyl bromide for the same exposure period, in the presence of mechanical means of gas recirculation throughout the grain mass, showed such measures to also be fully effective. Under such conditions, the required concentration of the fumigant was obtained throughout the grain mass by the end of the first 12 to 15 hours of exposure and was maintained for 48 hours, with the occasional addition of more methyl bromide.

A third test, made under conditions similar to the second but without the use of recirculation equipment showed the results to be equally effective, though required gas concentration in the grain mass was not reached until after the first 18 to 24 hours exposure. This cut the margin for holding 2 pounds for 24 hours pretty close to the termination of the 48-hour exposure period required and on occasion it was necessary to extend the latter period for several hours. This presented a difficult situation in that experience had demonstrated that it was next to impossible to find 5 consecutive days under California conditions without an afternoon or night wind of 25 to 50 miles per hour. On occasion such winds played havoc with the cover, sometimes resulting in total loss of the fumigant. For that reason, primarily, it has been recommended that some form of gas recirculation be provided where bulk grain or seed is involved in order to assure obtaining and maintaining the required gas concentrations well within the 48-hour exposure period.

PREVENTING WIND DAMAGE TO COVER.—The wind problem has been partly solved by providing exhaust fans used in removing, prior to introduction of the fumigant, any surplus air captured during the covering of the building or resulting from heat expansion during the day, either of which was otherwise responsible for huge blisters or balloons which aided wind tear. It has even been found possible during fumigation to remove such air recaptured during repairs, without significantly affecting the gas concentration.

RELEASE OF RESIDUAL GAS.—Safe release of the high volume of fumigant, following completion of the exposure period, has presented a problem, particularly in highly congested areas. Each pound of methyl bromide introduced under the cover represents 4 cubic feet of gas or 20 cubic feet per 1,000 cubic feet of space. This has amounted to as much as 80,000 cubic feet in one instance with approximately one-half that amount remaining under the gastight covers at the end of the 48-hour exposure.

In conducting the tests referred to it was important that this residual gas be released immediately after the 48-hour exposure was completed. In so doing it was found hazardous to make such releases at low temperature or during periods of high humidity. Under such conditions the gas was found to hug the ground and move out into adjacent areas at concentrations far above tolerance. However, it was found that if release could be accomplished during the heat of the day, this problem could be avoided.

In the event field operations did not permit timing so that release would occur between 10:00 A.M. and 2:00 P.M., the extended exposure required to meet that condition would be a benefit rather than adverse. Where local conditions have made it advisable, even under this timing, exhaust fans have been used to make the initial withdrawals, using force to dispel the fumigant upwards.

TREATMENTS OF SURFACE AREA.—While the adult beetles do not fly, under population pressure the larvae are forced outside of infested structures and may be found in numbers under any ground debris that may occur within 50 to 100 feet. To meet this problem it has become general practice to rake all such surface debris toward the building and include it under the fumigation cover. The areas thus cleaned are sprayed repeatedly up to the time of actual fumigation, using 5 pounds of malathion in 100 gallons straight diesel oil. Where blacktop surfacing is involved the oil is replaced with water, as the blacktop and diesel are not compatible. Here again we have volume use, 7,000 gallons having been required in treating the surface area adjacent to a single premise.

ERADICATION RESULTS ENCOURAGING.—The over-all situation from the standpoint of eradication is not nearly as serious as it may sound. It is true that 230 premises have been found infested but this represents less than one per cent of the potential premises lending themselves to infestation, which have been inspected to date. In recent weeks, federal-county inspection, in which every road in a given area is being ridden out and every farm visited and grain storage examined, has recorded not more than two infested premises per thousand examined. Also, even though the initial inspections showed infestation present in 18 of the state's 58 counties, with the extremes over 800 miles apart, most of these counties have been removed

from the infested list by approved fumigation of the limited number of widely scattered infested premises involved. The balance still to be treated, representing the larger number, are restricted primarily to three counties, namely, Imperial, Kern and Riverside in that order.

As a result of the tests mentioned, the fumigation of 101 premises representing close to 37,000,000 cubic feet has been effectively completed to date. These represent practically all of the larger warehouses so far found infested and amounts to approximately two-thirds in number and three-fourths in volume of all known infested premises in the state. Rapid treatment of the 129 remaining premises is scheduled, with a full inspection program to be continued indefinitely.

EXCEPTIONS NECESSARY TO FUMIGATION PROGRAM.—Some premises do not lend themselves to fumigation. In such cases any reasonable measure or measures, applied under official supervision, are recognized subject to delayed release from quarantined status. In such instances release is predicated on negative findings resulting from official inspections made at 180-, 90-, and 90-day intervals, or a period of a full year from the time of application of the measures used. The final inspection must be made by federal representatives.

COST OF ERADICATION PROGRAM.—The cost of the measures being applied in this sincere suppressive effort, as applied in all infested states, seems prohibitive in itself. In California at least $750,000 has been or will be expended by the close of the current fiscal year by the State Department of Agriculture and another $100,000 contributed by the U. S. Department of Agriculture in the form of materials and survey personnel. However, the crops being protected by these measures returned $221,000,000 to growers in 1954. The cost to date is therefore less than one-half of one per cent of that total. If accepted as an established species, infestation would be gradually extended, possibly even to other states and continue indefinitely, with a proportionate increase in the high cost of control, exclusive of the loss from the attack of the beetle.

LOSSES FROM ATTACK.—What the loss could be from the presence of the khapra beetle is graphically demonstrated in the case of the infested premise at Alpaugh, previously discussed, in which the 1954 crop was more heavily infested than the 1953 crop, in spite of the extensive and expensive spray measures applied. In April 1954, this building and its contents were fumigated using the eradicative schedule. But even though the measures used were found to be completely effective as far as mortality of all stages of the beetle present was concerned, prior serious damage had already been done. The seasonal movement from this 3700-ton mass showed 20% loss in weight from larval feeding of the khapra beetle. This single instance should be of sufficient significance to demonstrate the pest importance of the species.

In addition to direct loss from attack, all infested premises are kept under quarantine with treatment of all host grain required as a condition of movement. Such premises have been ruled unacceptable for government grain storage which in many instances, has previously been a major source of income. Also, others are hesitant to deal with infested premises regardless of the safeguard measures imposed. Owners are therefore most anxious to have infested premises immediately treated and officially released from quarantine status, and thus eliminate these indirect sources of loss.

CONCLUSIONS.—The most obvious question is, "Can eradication be accomplished?" It certainly cannot unless the attempt is made. The economics of the situation would most certainly seem to justify the attempt. The possibility of accomplishment would seem to be now restricted only by the degree to which all existing infestation can be found, and that to which further spread can be restricted during the period of the attempt.

Corn Earworm Populations on Sweet Corn in Central Florida During the Growing Seasons of 1954 and 1955[1]

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The effectiveness of insecticide applications for control of the corn earworm, *Heliothis zea* (Boddie), is dependent upon a number of factors. The intensity of earworm populations is often the most important of these factors (Chamberlain 1951, Garman & Jewett 1914, Wilson 1955). Wene & Blanchard (1955) recommend that in areas where infestations are likely to be very severe, insecticide applications should be started at least 2 to 3 days before the first appearance of silks in the field. During the 1954 and 1955 growing seasons, a survey of commercial sweet corn fields in the vicinity of Sanford and Zellwood Florida was conducted to study the seasonal abundance and distribution of corn earworm eggs and damaged ears in individual fields.

METHODS.—The survey was started both seasons when the early-planted commercial fields began silking and successive fields were visited throughout the growing season. Each of the survey fields was visited when it was in full silk. Egg counts were made by examining 20 silk masses on adjacent plants at 9 positions, making a total of 180 observations in each field. Three positions in the second row at the margin, 3 positions approximately one-fourth of the distance from the margin and 3 positions near the center row of the field, were examined. Because of the variation in the size of the fields, the distance from the margin of the field to the other two locations in the field varied considerably from field to field. Figure 1 illustrates the approximate location of each of the above described positions. Records were made of the location of the field, the insecticide being used for earworm control, the size of the field and the number of eggs found on each

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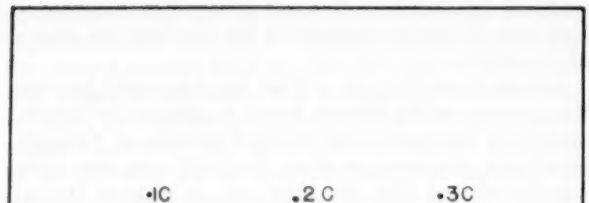

FIG. 1. Illustrating the approximate location in each field where counts of earworm eggs and infested ears were made.

silk mass. Prior to the time for harvest (14 to 18 days after the egg count), the same fields were visited and infested-ear counts were made at about the same positions where the egg counts had been made. The husk was cut off about 1 inch from the tip of the ear and the cut surface examined for the discolored area, indicating that an earworm larva had passed down the silk channel. Each ear that showed such a discolored area was recorded as being infested. All of the fields visited were treated with insecticides for earworm control at 48-hour intervals during the silking period.

DISCUSSION.—A summary of the data for the two seasons' survey is presented in tables 1 and 3. As was expected, the data for both seasons in the Sanford area shows that the population during the early part of the season was low and gradually increased as the season advanced. In the Zellwood area, the populations for both seasons were very low and remained low through the whole growing season. Because no significant differences were indicated, the detailed data are omitted from the tables. The Zellwood area is a peat area bordering the northern and northeastern shores of Lake Apopka. During the fall and winter months none of the preferred host plants of the corn earworm are growing in this section.

The temperatures in this area during cold periods average 6 to 10 degrees lower than in the Sanford area (Sherouse 1955). Sweet corn planted before the first of March is frequently killed by frosts occurring during the first part of March. The Florida State Market Bureau reported an acreage of sweet corn of 3,850 for 1955. The absence of preferred host plants during the fall and winter months and the relatively lower winter temperatures experienced in this area tend to reduce the early season populations to a low point. The dilution of the large acreage is apparently a factor in keeping the population in any one field low during the 5-month growing season, which ends during the first part of July.

In tables 2 and 4 the data given in tables 1 and 3 are rearranged to show the field distribution of the eggs and infested ears. These figures were converted to per cent of silks and ears infested; then the percentages were expressed as arc sign transformations (Snedecor 1946) for an analysis of variance test. These analyses gave the following F values for the Sanford data: 1954, F value 5.20, observed F at 5% level 3.44; 1955, F value 3.59, ob-

Table 2.—Distribution of corn earworm eggs and infested ears in commercial sweet corn fields during the spring of 1954. Sanford, Florida.

Field No.	Number Silk Masses Infested at Locations			Number Ears Infested at Locations		
	Edge	One-fourth Way in	Center	Edge	One-fourth Way in	Center
1	1	0	0	0	0	0
2	0	0	0	1	2	2
3	6	10	6	2	0	0
4	0	0	0	2	1	1
5	1	1	3	0	1	0
6	0	0	1	1	0	0
7	2	0	1	1	3	3
8	7	5	7	4	2	2
9	0	0	1	0	1	1
10	14	4	4	33	13	15
11	11	2	2	16	7	8
12	23	10	12	11	14	3
13	22	14	13	—	—	—
Totals	87	46	52	71	44	35

Table 1.—Results of survey of commercial sweet corn fields during 1954 season. Sanford, Florida.

Field No.	Size of Field (Acres)	Earworm Eggs on Silks				Earworm Larvae in Ears		
		Date Examined	Total Number Eggs Found	Silk Masses Infested		Date Examined	Ears Infested	
				Number	Per Cent		Number	Per Cent
1	10	4/19	1	1	0.55	5/1	0	0
2	20	4/19	0	0	0	5/1	5	2.78
3	8	4/23	33	22	12.22	5/4	3	1.67
4	15	4/23	2	2	1.11	5/4	4	2.22
5	10	4/27	8	5	2.78	5/11	1	0.55
6	10	4/27	1	1	0.55	5/11	1	0.55
7	7	4/27	3	3	1.67	5/11	7	3.89
8	8	5/3	20	19	10.56	5/13	8	4.44
9	10	5/3	1	1	0.55	5/11	2	1.11
10	5	5/3	28	22	12.22	5/13	61	33.89
11	15	5/3	16	15	8.33	5/13	31	17.22
12	15	5/11	68	45	25.00	5/24	28	15.56
13	2	5/13	71	49	27.22	—	—	—

Table 3.—Results of survey of commercial sweet corn fields during the spring of 1955. Sanford, Florida.

Field No.	Size of Field (Acres)	Earworm Eggs on Silks				Earworm Larvae in Ears		
		Date Examined	Total Number Eggs Found	Silk Masses Infested		Date Examined	Ears Infested	
				Number	Per Cent		Number	Per Cent
1	10	4/15	20	15	8.33	5/1	32	17.78
2	30	4/25	9	8	4.45	5/10	12	6.67
3	15	4/26	8	8	4.45	5/10	1	0.55
4	10	4/29	24	21	11.67	—	—	—
5	2.5	4/29	17	12	6.67	5/16	3	1.66
6	1.5	5/2	8	7	3.89	5/16	14	7.22
7	10	5/6	31	27	15.00	5/20	35	19.44
8	10	5/23	43	38	21.11	6/6	156	86.67
9	12	5/23	87	58	32.22	—	—	—
10	6	5/23	181	93	51.67	6/6	118	65.56
11	8	6/2	80	51	28.33	—	—	—
12	30	6/6	18	15	8.33	—	—	—
13	15	—	—	—	—	6/3	150	83.33

Table 4.—Distribution of corn earworm eggs and infested ears in commercial sweet corn fields during the spring of 1955. Sanford, Florida.

Field No.	Number Silk Masses Infested at Locations			Number Ears Infested at Locations		
	Edge	One-fourth Way in	Center	Edge	One-fourth Way in	Center
1	13	2	0	13	17	2
2	6	2	0	4	5	3
3	0	5	3	0	1	0
4	5	10	6	—	—	—
5	1	2	9	0	2	1
6	2	2	3	2	6	6
7	9	10	8	18	12	5
8	16	12	10	32	51	53
9	19	21	18	—	—	—
10	29	36	28	36	49	33
11	30	20	1	—	—	—
12	4	7	4	—	—	—
13	—	—	—	48	50	52
Totals	134	129	90	153	193	155

served F at 5% level 3.40. These F values are interpreted to mean that the high earworm populations found at the margins of the fields are significantly different.

ACKNOWLEDGMENTS.—The author is indebted to Mr. Howard Van Pelt of the State Plant Board who assisted in the 1954 survey in the Sanford area. The author is indebted to Dr. W. D. Hanson for advice on the field methods used and suggestions on the statistical treatment of the data. Drs. D. O. Wolfenbarger and E. G. Kelsheimer read the manuscript and made helpful criticisms.

SUMMARY.—Results of two seasons' survey of corn earworm populations in commercial sweet corn fields are presented. In the Sanford area, the populations increased as the seasons advanced and the populations at the margins of the fields were much higher than in other parts of the fields. In the Zellwood area, the populations were very low throughout both growing seasons.

REFERENCES CITED

Chamberlain, W. F. 1951. Pre-silking sprays to control the corn earworm and fall army worm. Jour. Econ. Ent. **44**(4): 590–2.

Garman, H., and H. H. Jewett. 1914. The life history and habits of the corn earworm. Ky. Agr. Expt. Sta. Bull. **187.**

Sherouse, R. T. 1955. Annual Report Horticultural Protection Service, Orlando District, U. S. Weather Bureau. Mimeographed.

Snedecor, G. W. 1946. Statistical Methods, Fourth Edition. Iowa State College Press. pp. 449.

Wene, G. P., and R. A. Blanchard. 1955. Relation between sweet corn silking and earworm infestation in the lower Rio Grande Valley of Texas. Fla. Ent. **38**(1) 13–6.

Wilson, J. W. 1955. Control of the corn earworm in Florida. Jour. Econ. Ent. **48**(4): 442–4.

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The Synergistic Activity of Piperonyl Butoxide Applied at Different Intervals to Pyrethrum-Treated House Flies[1]

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Chemicals capable of increasing the toxicity of pyrethrins to insects have been examined extensively by many investigators. These compounds, known as pyrethrum synergists, have been shown to activate markedly the various components of the pyrethrum complex, but, show less activity in combination with the allylic homologue of cinerin I, allethrin (Incho & Greenberg 1952). The most outstanding pyrethrum synergists contain the methylenedioxyphenyl group (Synerholm & Hartzell 1942, Synerholm *et al.* 1947, Wachs 1947), the biological importance of which was demonstrated first by Haller *et al.* (1942 a,b). Although these compounds are classified as insecticides, their relatively low toxicity to insects negates their use in this capacity.

Mode of action studies on pyrethrum synergists have revealed the complex action of these compounds. Wilson (1949) demonstrated that pyrethrum and the synergist could be separated spatially when applied to house flies without diminishing the synergy. In one of the most extensive studies of synergists, Chamberlain (1950) reported that piperonyl butoxide partially inhibited the enzymatic hydrolysis of pyrethrins in cockroach extracts. Since known esterase inhibitors were ineffective as *in vivo* synergists, it is probable that additional mechanisms are operating to produce the overall synergistic response. The temporal separation of the insecticide and synergist was described first by Lindquist *et al.* (1947) who showed that application of the synergist after the pyrethrum produced a response equal to application of the materials simultaneously; the reverse of the order of application produced no mortality when the interval exceeded 30 seconds. In these tests the house flies were exposed to sprays; consequently, the amount of both materials that a fly received was indeterminate.

We have studied various aspects of pyrethrum synergism, one of which was the activity of piperonyl butoxide when applied after the insecticide. Treatment of individual flies demonstrated that this synergist has an appreciable amount of activity when applied after the insecticide.

METHODS AND MATERIALS.—Female house flies,[3] 3 to 4 days old, were used for all tests. Four groups of 20 flies in duplicate were used in each experiment. The flies were fed on an evaporated milk-water mixture (1:3) until the time of treatment. After application of the chemicals, the insects were placed in pint Mason jars for knockdown and mortality determinations. During the 24-hour observation period, the flies were fed a 2% solution of sucrose. The treated insects were kept at room temperature (26 to 27° C). All materials were applied to the flies by means of a tuberculin syringe-micrometer assembly. Topical applications were made to the prothorax of CO_2-anaesthetized flies. Synergist emulsions were introduced into the thorax of the fly using the above assembly. The needle was not modified.

The pyrethrins were purified by chromatographing the technical material on carbon. The piperonyl butoxide[4] was a middle fraction of the distillate obtained from the technical material as described by Miller *et al.* (1952).

Topical applications were made as 1 μl acetone solutions. When injected, the synergist was emulsified in insect saline (Waterhouse 1947) in a Waring blender with "Tween 80"[5] (a mixture of polyoxyethylene sorbitan mono-oleate), a non-toxic emulsifier. One microliter of synergist-fortified saline was injected into each fly.

RESULTS AND DISCUSSION.—The ability of piperonyl butoxide to synergize pyrethrum in insects previously treated with this insecticide is shown clearly by the data presented in table 1. Both controls of pyrethrum and synergist received a second application of acetone. Within the first 3 hours of separation of the pyrethrum and synergist knockdown is great, but it decreases rapidly after this time. Since the optimal ratio of synergist to pyrethrum is regarded as 10:1 when the materials are applied simultaneously, a group of experiments was performed in which this ratio was employed. When the level of synergist was reduced, mortality substantially decreased (table 2). It is interesting to note that mortality only resulted in those groups of flies that were immobilized in the first hour after treatment with the synergist.

Table 1.—The knockdown and 24-hour mortality of house flies topically treated with 0.1 μg./fly of pyrethrum followed by 10 μg./fly of piperonyl butoxide (synergist) at various time intervals.

TREATMENT	HOURS BETWEEN PYRETHRUM AND SYNERGIST	PER CENT KNOCKDOWN AFTER ½ HOUR	PER CENT MORTALITY AFTER 24 HOURS
Pyrethrum and synergist	1	100	100
	2	95	95
	3	90	95
	4	20	90
	5	20	95
	6	0	85
	7	0	70
	8	0	45
Pyrethrum	—	0	0
Synergist	—	0	5

The ability of the synergist to activate the pyrethrum enough to kill the house fly is dependent partially on the concentration of pyrethrum in the insect (Wilson 1949). Since the house fly may be regarded as capable of actively

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[2] Present Address: Quartermaster Research and Development Command, Natick, Massachusetts.

[3] A strain highly resistant to DDT, but showing no increased resistance to pyrethrum.

[4] Kindly supplied by A. C. Miller of the Gulf Research & Development Company, Pittsburgh 30, Pennsylvania.

[5] Atlas Powder Company.

Table 2.—The knockdown and 24-hour mortality of house flies topically treated with 0.1 μg./fly of pyrethrum followed by 1 μg./fly of piperonyl butoxide (synergist) at various time intervals.

Treatment	Hours Between Pyrethrum and Synergist	Per Cent Knockdown After ½ Hour	Per Cent Mortality After 24 Hours
Pyrethrum and synergist	1	70	25
	2	70	35
	3	60	40
	4	30	30
	5	20	15
	6	0	0
	7	0	0
	8	0	0
Pyrethrum	—	0	0
Synergist	—	0	0

Table 3.—The knockdown and 24-hour mortality of house flies topically treated with 0.1 μg./fly of pyrethrum followed by 1 μg./fly of injected piperonyl butoxide (synergist) at intervals.

Treatment	Hours Between Pyrethrum and Synergist	Per Cent Knockdown After ½ Hour	Per Cent Mortality After 24 Hours
Pyrethrum and synergist	1	90	65
	2	95	60
	3	85	70
	4	20	60
	5	0	30
	6	0	35
	7	0	30
	8	0	30
Pyrethrum	—	0	0
Synergist	—	0	5

metabolizing the applied pyrethrum, the amount of pyrethrum available for synergy will diminish as the temporal separation of the two materials is increased. Under these circumstances the ability of the synergist to increase the toxicity of pyrethrum in treated flies would be contingent on the availability of the synergist at those sites critical to activation. Since the rate of penetration of materials across the cuticle is concentration-dependent, among other things, it would be expected that higher concentrations of topically applied synergist would be more effective in activating pyrethrum present internally in the insect. If this hypothesis is correct, it would be expected that injection of the synergist would enhance the toxicity of the pyrethrum more effectively than the same amount applied topically. Since the total response of a topically applied 10:1 proportion of synergist to pyrethrum mixture was poor compared to 100:1 mixture, mortality responses were determined after injection of the synergist into pyrethrum-treated flies (10:1).

Data given in table 3 clearly illustrate that a given amount of piperonyl butoxide when injected potentiates the same amount of pyrethrum substantially more than the same amount of synergist when topically applied to treated flies after various periods of time.

The ability of the synergist-pyrethrum interaction to produce a good knockdown appears to be associated frequently with high mortality. Regardless of the means of synergist application, no immediate knockdown reaction resulted after a 5-hour temporal separation of the pyrethrum and synergist; however, maximum knockdown occurred within the first 3 hours of separation.

SUMMARY.—The ability of piperonyl butoxide, a pyrethrum synergist, to enhance the toxicity of pyrethrum when applied after the insecticide was demonstrated. A high ratio of synergist to insecticide produces substantial kills even if the synergist is applied 8 hours after the insecticide. At similar concentrations, injected applications of the synergist are more efficient than topical applications when the synergist is applied to pyrethrum-treated flies. At normal concentrations of pyrethrum-synergist, it appears that knockdown is an indication of ensuing mortality in a treated population.

REFERENCES CITED

Chamberlain, R. W. 1950. An investigation on the action of piperonyl butoxide with pyrethrum. Amer. Jour. Hygiene **52**: 153–93.

Haller, H. L., F. B. LaForge, and W. W. Sullivan. 1942a. Some compounds related to sesamin: their structures and their synergistic effect with pyrethrum insecticides. Jour. Org. Chem. **7**: 185–8.

Haller, H. L., E. R. McGovran, L. D. Goodhue, and W. W. Sullivan. 1942b. The synergistic action of sesamin with pyrethrum insecticides. Jour. Org. Chem. **7**: 193–4.

Incho, H., and H. Greenberg. 1952. Synergistic effect of piperonyl butoxide with the active principles of pyrethrum and allethrolone esters of chrysanthemum acids. Jour. Econ. Ent. **45**: 794–7.

Lindquist, A. W., A. H. Madden, and H. G. Wilson. 1947. Pre-treating house flies with synergists before applying pyrethrin sprays. Jour. Econ. Ent. **40**: 426–7.

Miller, A. C., J. P. Pellegrini, Jr., A Pozefsky, and J. R. Tomlinson. 1952. Synergistic action of piperonyl butoxide fractions and observations refuting a pyrethrins-butoxide complex. Jour. Econ. Ent. **45**: 94–7.

Synerholm, M. E., and A. Hartzell. 1945. Some compounds containing the 3,4-methylenedioxyphenyl group and their toxicities toward house flies. Contrib. Boyce Thomp. Inst. **14**: 78–89.

Synerholm, M. E., A. Hartzell, and V. Cullmann. 1947. Pyrethrum extenders. Contrib. Boyce Thomp. Inst. **15**: 35–45.

Wachs, H. 1947. Synergistic insecticides. Science **105**: 530–1.

Waterhouse, D. F. 1947. Toxicity of DDT, *Lucilia* (Diptera) larvae. Counc. Sci. Ind-Res. (Australia) Bull. **218**: 17–8.

Wilson, C. A. 1949. Piperonyl butoxide, pipreonyl cyclonene and pyrethrum applied to selected parts of individual house flies. Jour. Econ. Ent. **42**: 423–8.

Differential Reactions of Two Biotypes of the Corn Leaf Aphid to Resistant and Susceptible Varieties, Hybrids and Selections of Sorghums[1]

JEAN JACQUES CARTIER[2] AND REGINALD H. PAINTER[3,4]

The corn leaf aphid, *Rhopalosiphum maidis* (Fitch), is a serious pest of sorghums. Its presence in great numbers can cause direct damage through the loss of sap. Its feeding in the whorl may hinder the exsertion of the head from the boot. In some circumstances the seed gathered following a servere infestation is low in vitality and of poor quality. Losses as great as 33% in weight and 50% in volume were cited by McColloch (1921). The honey dew secreted by the aphids reduces the shedding of pollen, attracts the corn earworm moths, and favors the growth of fungi. Chloratic areas and purple discolorations on leaves and leaf sheaths are associated with heavy infestations of this insect. These facts, together with the practical difficulty of chemical control, were responsible for beginning the research project on resistance of sorghums to the corn leaf aphid.

The biology of the corn leaf aphid was last reviewed by Wildermuth & Walter (1932). With the exception of about 40 males obtained in their rearing experiments at Tempe, Arizona, between 1914 and 1924, and a few males occurring in these experiments, no sexual forms have been reported. The reproduction in nature appears to be exclusively parthenogenetic. Wintering in the latitudes south of northern Texas, the alate viviparous females are believed to migrate northward as the weather becomes favorable in the spring. In 1954 and 1955, the Canadian Insect Pest Review reported very alarming outbreaks of the aphid on late barley in the central and eastern provinces of Canada. Those are the most northern important infestations known for the species.

The world literature on insect resistance in crop plants has been reviewed by Painter (1951). In analyzing resistance, it has been found to be of maximum usefulness to divide the phenomena of resistance, as seen in the field, into three bases or mechanisms: *preference* or *non-preference, antibiosis*, and *tolerance*.

On corn, the means of expressing the relative resistance of varieties or inbred lines or hybrids have been the degrees in the severity of attack by the aphid. Huber & Stringfield (1942) and Walter & Brunson (1940) reported marked differences in the damage caused to inbred lines or hybrids. The inheritance of resistance to the corn leaf aphid was demonstrated by Snelling *et al.* (1940) and Viale (1950). The latter found that different planting dates or experimental fertilization of corn plots with nitrogen, phosphorus, and potash, in different combinations, rates, and methods of application did not produce noticeable change in the resistance or susceptibility to the aphid of two strains of corn, K 1639 and DeKalb 847. Resistant as well as susceptible strains maintained their respective genetic character under extreme variations of environmental and biological conditions. The character for resistance seemed to act as a recessive. A few modifying factors appeared to be present.

On sorghums, McColloch (1921) was the first to report on resistance to the corn leaf aphid. He expressed his results by stating that among 17 varieties the percentage of plants showing appreciable injury varied from 3.1% in the case of Sudan grass to 96.5% for feterita. Howitt & Painter (1956) carried on an extensive study of the sources and nature of resistance to the corn leaf aphid in sorghum varieties. He found a wide range of infestation among the 595 varieties, involving 13,662 plants studied. Sudan-type sorghums consistently and without exception demonstrated a relatively high level of resistance. Resistance resulted from non-preference and antibiosis. He increased the seed of a single plant (428-1) of the variety Piper Sudan grass that possessed a high degree of resistance approaching immunity. Taller kafir varieties and milos were predominantly intermediate in resistance, while combine-type sorghums were found to be either intermediate or highly susceptible to the corn leaf aphid.

The occurrence of biological races in insects has been recognized in many orders, as reported by Thorpe (1930, 1940) and Smith (1941). Painter (1951) reviewed the studies on biological races of the grape phylloxera (*Phylloxera vitifoliae* (Fitch)), European corn borer (*Pyrausta nubilalis* (Hbn.)), hessian fly (*Phytophaga destructor* (Say)), pea aphid (*Macrosiphum pisi* (Harr.)), and greenbug (*Toxoptera graminum* (Rond.)). So far as known, no biological races of the corn leaf aphid have ever been reported in the literature.

Following Howitt's studies, the problem was developed along these lines:

1. The biology of the insect, especially the possibility of biological races in relation to resistance.
2. The effects of resistant and susceptible varieties, hybrids and selections of sorghums on the biology of the aphid.
3. The inheritance of resistance to the aphid in sorghums.
4. The production of suitable resistant sorghums, specifically the transfer of resistance from Sudan grass sorghums to combine-type sorghums.

MATERIALS AND METHODS.—The experiments were conducted in a greenhouse where the maximum temperatures varied with seasonal changes and daily periods of sunlight. The minimum night temperatures were maintained at approximately 60° F. during the winter. Where temperature was believed to be of prime importance as a

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basis of comparison between consecutive experiments, the summation of the degree-hours was computed from thermograph charts. The sum of all the hourly temperatures divided by the number of times they were recorded gave the mean temperature for the duration of a test. Natural light conditions prevailed except from late spring until early fall, during which period the glass roof had to be coated white. No artificial illumination was used. The humidity was kept at a relatively high level by wetting the floor of the greenhouse daily. All studies were made with general or selected populations of the corn leaf aphid.

BIOLOGICAL RACES.—In the fall of 1953, a preliminary test was set up to study the biology of some small aphids which had developed on Piper Sudan grass 428–1, the sorghum plant normally highly resistant to the corn leaf aphid. These preliminary results indicated that the small aphids could reproduce better on Piper 428–1 than the other corn leaf aphids collected at random. In November 1954, the investigations were resumed and the behavior, the weight of adults, and the rate of reproduction of two lines of aphids of known origin were studied. From day to day the evidence of two different biotypes became sufficient to confirm the earlier observations. The two lines were called biotype KS-1 and biotype KS-2, and their origin is described as follows:

Biotype KS-1. A line originating from a large parthenogenetic alate female, weighing 0.458 mg. and isolated from the general population by collecting in the greenhouse on November 11, 1954, from a colony on a White Martin sorghum plant.

Biotype KS-2. A line originating from a small parthenogenetic alate female, weighing 0.168 mg. and isolated from the general population by collecting in the greenhouse on November 11, 1954, from a Greenleaf Sudan grass plant.

The general population had come from many collections of aphids made on corn and sorghum in 1954. No morphological characters of specific nature have been found so far separating the two biotypes, and they were both identified as *Rhopalosiphum maidis* (Fitch) by Louise M. Russell, of the Entomology Research Branch U. S. Department of Agriculture. Their occurrence and distribution in nature is not known. In studying their reactions to susceptible and resistant sorghums, they have been differentiated qualitatively and quantitatively as is indicated in this paper.

PREFERENCE STUDIES.—A preference test in the greenhouse consisted of exposing the sorghum seedlings to the winged aphids only. From two lateral benches where a population was constantly maintained on White Martin, a susceptible variety, the aphids could fly to the central benches and visit or settle on the plants of their choice. As seen from figure 1, the central area was covered by replications of randomized rows of seedlings. They were kept covered with a cheese cloth until they had reached a height of 4 to 6 inches. Every 2 or 3 days afterward, each plant was examined and the items were recorded in the following manner: W=a winged aphid present, alone; n=a small nymph; N=a large nymph; A=an apterous adult female; C=a colony. The primary infestation always began in the whorl. If a colony was established, i.e., two or more nymphs, it was immediately removed by cutting the central leaf at the lowest possible level. This technique did not injure the plants which kept growing and were colonized in some cases more than one time or simply visited by one or more females that subsequently flew away. After each period of data recording, the flats were rotated on the benches to change their positions. When the number of colonies recorded in the check (the most preferred) nearly equalled the number of plants, the experiment was stopped. The results were analyzed in the following ways:

FIG. 1.—Disposition of the material during a preference test in a greenhouse. The central area was occupied by the experimental flats. In the foreground and the background were located the cultures of the corn leaf aphid from which the winged females could fly and infest the seedlings in the central area. Manhattan, Kansas. 1955.

Number of infestations per plant. The total number of colonies divided by the number of plants in the replication.

Number of winged aphids per plant. The total number of observed alate viviparous females divided by the number of plants in the replication.

When studying an F_2 population segregating for preference, the number of infestations per plant as expressed for varieties does not show the proportions of the population segregating for preference and non-preference. It was found convenient to pool the data from the replications and calculate the percentage of plants that were not colonized or colonized once, twice, or more. The classes of preference ranged from 0 to 3. For plant breed-

ing screening purposes, the class 0 was kept as a source of non-preferred genotypes and subsequently tested by progeny tests.

During the experiments, it was found desirable to see if the biotype KS-1 and the biotype KS-2 of the corn leaf aphid could demonstrate differentially their preference towards White Martin (preferred) and Piper 428–1 (non-preferred) and the F_1 hybrid White Martin $\times$Piper 428-1. The behavior and the responses of the biotypes to these hosts were not known. The inheritance of preference might also be studied by use of the material at hand.

Due to the impossibility of caging three plants together and following the reactions of the insects in a large glass cage, the aphids were brought into the presence of leaf cuttings of these hosts within a small transparent plastic box. After some preliminary tests with boxes of several shapes and sizes, one with the following inside dimensions was adopted: $2\frac{1}{16}'' \times 2'' \times \frac{5}{16}''$. The cover had two hinges and a lock. Leaf strips of $1\frac{3}{4}'' \times \frac{3}{8}''$ were cut from homologous parts of the desired plants and fastened to the inside of the box with narrow bands of adhesive tape set along the leaf edges. In other preliminary tests, apterous adults and nymphs did not prove to be as responsive as did the winged aphids. The aphids were introduced into the box and their reactions observed through the pastic box. One box containing three strips of different plant material and 10 winged aphids formed one replication. Three replications for each biotype were tested simultaneously under different environmental conditions. After 1 hour of contact with their new environment, the aphids were showing their optimum pattern of preference for the leaf strips. The results follow later.

ANTIBIOSIS STUDIES.—As in the preference tests, the seedlings were arranged in randomized rows in four replications. The seedlings, 4 to 6 inches tall, were individually infested twice, a few days apart, with apterous aphids. When the susceptible check had a heavy population of aphids, direct counts of the total number of aphids were made on each plant. The data from the four replications were analyzed and the varieties were classified according to their mean infestation per plant expressed as *aphids per plant*. With material segregating for antibiosis, the counts of *aphids per plant* were grouped in classes of 5 or 10 aphids per plant. The classes were arbitrarily determined to give a reasonable distribution that lent itself to statistical analysis. For screening and plant breeding purposes, the lowest classes of infestation were selected as sources of possible resistant genotypes. Progeny tests confirmed the usefulness of this technique as did subsequent antibiosis cage tests. This method of grading expresses also preference in part because the aphids were still free to leave a plant. But by using apterous aphids and a repeated initial infestation, it is assumed that the results expressed largely the antibiosis and very little of the preference qualities of the plants.

The antibiosis of a particular host plant was studied by rearing the aphids on individual seedlings or on parts of mature plants. The cage is illustrated in figure 2. The cage consisted of a tube of transparent Visking dialyzing material approximately 1 inch in diameter. Each cage had two fine mesh, nylon windows 1 square inch, 2 inches from the ends, on opposite sides. A convenient length of tubing for sorghum seedlings was 14 inches. The upper part of the cage was closed with a double fold of the material and a paper clip. After having placed a small quantity of absorbent cotton as a seal around the stem, the lower part of the cage was folded at 45° and a paper clip was put across the fold. The cage was held in vertical position by a string that was attached to the upper clip and to a wire support.

Each cage was infested with one female of a known life history stage, and the total progeny obtained after a given period was counted. The total number divided by the number of days gave a mean increase of *aphids per day*. Whenever possible, the original alate and apterous adults and those in the progeny of the individual females were weighed on a Roller-Smith micro-torsion balance having a capacity of 5 mg. and could be read to 0.002 mg. Notes were also taken on the behavior, the mortality of nymphs, and the preference in location for feeding on the host.

EVIDENCES OF THE INHERITANCE OF PREFERENCE.—To study the responses of the biotype KS-1 and the biotype KS-2 of the corn leaf aphid in the presence of (1) a preferred host, White Martin; (2) a non-preferred host, Piper 428-1; and (3) the F_1 hybrid between these two

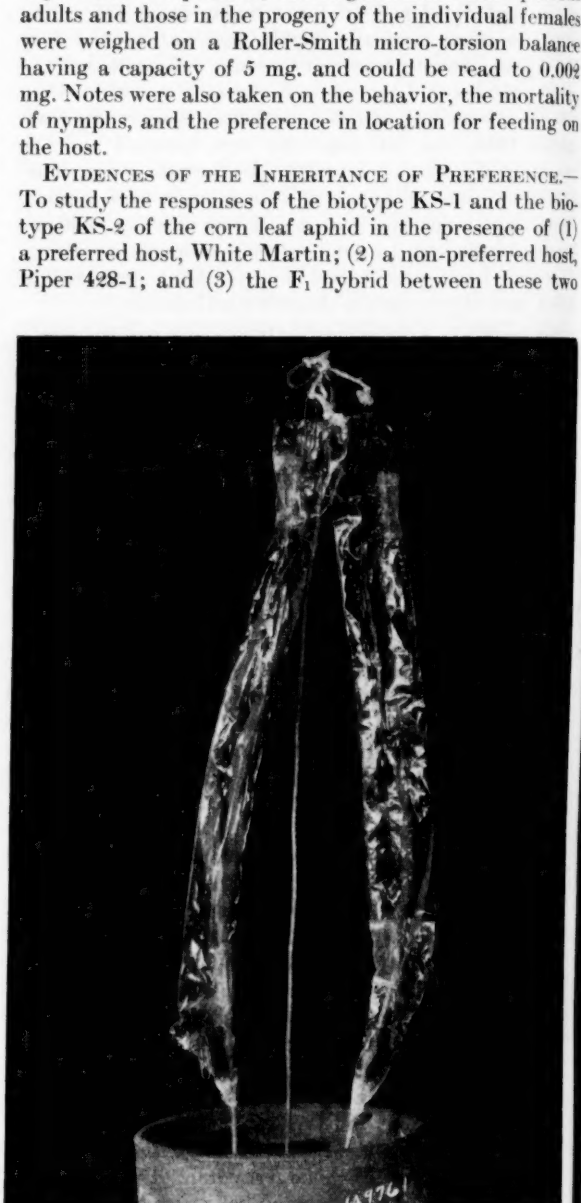

FIG. 2.—Rearing cage used in antibiosis tests. The cage was made of transparent Visking dialyzing tubing of an approximate diameter of one inch. Manhattan, Kansas, 1955.

hosts, three series of tests were made using the plastic boxes previously described. Each box contained three leaf strips, one from a preferred host, one from a non-preferred host, and one from the hybrid between the two. After the 10 winged females had been introduced into the boxes, they were all restless for about 20 minutes. Most of them seemed to look for an escape and apparently were not immediately attracted to the leaf strips. However, after 20 minutes, a pattern of preference began to show. Some nymphs were born and remained exactly where they were born for the whole period of the observations. After 1 hour, the pattern of distribution of the females had been determined. While the pattern remained constant for nearly 2 hours in some cases, the readings were taken after 1 hour. Two or three hours later, the tissues of the hosts were drying and most of the aphids were again restless. The grouping of the aphids on one leaf may, in part, have been a result of the attraction of aphids for each other similar to the "fly factor" discussed by Barnhart & Chadwick (1953). The repeated experiments with similar results indicate that basically a preference for particular varieties actually exists.

Three boxes for each biotype were tested for 1 hour in three repeated experiments. The results of the observations were grouped in table 1. The preference of the alate aphids for White Martin was highly significant. The number of nymphs born on White Martin, during the test hours, was also significantly higher than on the other hosts. Based on the number of adults settled or the number restless, and the number of nymphs born on the hosts, there were no significant differences between the biotypes.

Results of preference tests on the parental sources and the F_2 generation of five crosses of sorghums are presented in table 2. Four of these crosses had Piper and Piper 428-1 as one of their parents. In another cross, Oklahoma 130 Sudan grass, also a non-preferred host, was one of the parents. The results are expressed in percentage of preference which is the number of infestations per plant multiplied by 100. In all of the crosses except KS-1044×Piper Sudan grass, the F_2 generation as an average was preferred less than the most preferred parent of each cross. This tendency was the result of a higher percentage in non-preference, as seen in class 0, and of a lower percentage of preference, as seen in classes 1, 2, and 3. In White Martin×Piper, the class 1 of the F_2 generation expressed the summation of the tendencies of both parental populations and was higher than either of them. In the case of KS-1044 (4n)×Piper Sudan grass, it was doubtful whether the resistance was actually transferred to the F_2 generation.

EVIDENCES OF THE INHERITANCE OF ANTIBIOSIS.—Following the discovery of a selection of Sudan grass sorghum, Piper 428-1, resistant to the corn leaf aphid, the Department of Agronomy in cooperation with the

Table 1.—The preference of winged females of biotype KS-1 and biotype KS-2 of the corn leaf aphid for leaf cuttings of the parents and the F_1 hybrid of the cross between White Martin and Piper Sudan grass 428-1 in 1-hour tests in plastic boxes, Manhattan, Kansas, 1955.

Biotype	Test No.[a]	Temperature, (° F.)	Number of Females Settled on			Number of Restless Females	Number of Nymphs Born on			Totals
			W.M.[b]	F_1	P.		W.M.	F_1	P.	
KS-1[c]	1	75	4	1	0	5	2	0	0	2
			6	1	1	2	6	2	0	8
	2	84	2	1	0	7	0	0	0	0
			2	4	4	0	1	0	2	3
			5	1	1	3	0	0	1	1
	3	85	2	0	2	5	0	0	0	0
			5	0	1	4	1	0	0	1
			5	2	1	2	1	0	0	1
	Grand totals[d]		31	10	10	28	11	2	3	16
KS-2[c]	1	75	2	0	0	8	3	0	0	3
			4	1	1	4	5	1	0	6
			5	2	1	2	5	1	0	6
	2	84	3	3	1	3	0	0	0	0
			3	1	0	6	3	1	0	4
			3	1	3	3	0	0	0	0
	3	85	1	0	0	9	2	0	0	2
			1	1	4	4	2	0	0	2
			1	1	2	6	0	0	0	0
	Grand totals[d]		23	10	12	45	20	3	0	23

[a] Three replications of each test except No. 1 with KS-1. Ten females placed in each box except in the first replicate of Test No. 3 with KS-1 which had only nine.

[b] W.M. = White Martin. F_1 = Hybrid. P. = Piper Sudan grass 428-1.

[c] The hypothesis of differences between the biotypes based on the number of females settled, the number of restless females, and the number of nymphs born during the experiments was rejected by chi-square tests.

[d] The hypothesis of random distribution in the number of females and the number of nymphs was rejected by chi-square tests.

Table 2.—The preference of winged females of a general population of the corn leaf aphid towards the parental varieties and the F_2 generation of five crosses of sorghums. Manhattan, Kansas, March 29 to May 8, 1954, and November 20, 1954, to January 3, 1955.

Parental Source or Generation[a]	Percentage of Plants Colonized 0, 1, 2, 3 Times				No. of Seedlings	Total Per Cent of Preference	Winged Females Observed per Plant
	0	1	2	3			
White Martin[b]	1.3	41.3	46.6	10.8	75	98.7	0.20
F_2	8.5	58.0	30.8	2.7	848	91.5	.16
Piper 428-1-S1	27.6	53.9	15.8	2.7	76	72.4	.13
Oklahoma 130	76.7	23.3			60	23.3	.00
F_2	79.3	19.0	2.7		58	21.7	.03
Piper	87.9	12.1			58	12.1	.03
Oklahoma 130	76.7	23.3			60	23.3	.00
F_2	66.7	28.3	3.3	1.7	60	33.3	.05
Tift, FC 24834	50.0	41.0	9.0		56	50.0	.07
KS-1044 (4n)	47.4	33.9	18.7		59	52.6	.05
F_2	43.8	40.4	15.8		57	56.2	.19
Piper	87.9	21.1			58	12.1	.03
Tift, FC 24834	50.0	41.0	9.0		56	50.0	.07
F_2	55.2	39.6	3.4	1.8	58	44.8	.07
Piper	87.9	12.1			58	12.1	.03

[a] The F_2 generations in the table are accompanied by their parental sources above and below each of them.
[b] White Martin is a combine-type sorghum. The others are Sudan grass sorghums.

Department of Entomology started a breeding program to transfer the resistance of that selection to combine-type sorghums.

A population of aphids increased from biotype KS-1 was used in a first screening antibiosis test (March, 1955) on seedlings of White Martin, Piper 428-1-S1, and the F_2 generation of the cross White Martin×Piper 428-1. The infestations ranged from 0 to 75 aphids per seedling on White Martin. The distribution of infestation within each group tested was analyzed on the basis of classes of five aphids per plant, giving a spread over 15 classes on White Martin. The results are illustrated in figure 3 with the blocks on the left. In a second test (May, 1955) on White Martin, Piper 428-1-S1, the F_2 and F_3 generations of the cross under investigation, the infestation ranged from 0 to 150 aphids per plant. The other groups of seedlings were proportionately more infested. The results are represented in figure 3 by the blocks on the right. This increase in infestation over an equal period of time was probably the result of the higher temperatures, longer periods of sunlight, and better growing conditions in the plants, all of which favored the reproduction of the aphids. However, in analyzing the results on the basis of classes of 10 aphids per plant, the pattern of distribution in the classes of infestation was very nearly the same as in the first test. Since the relative characteristics of the populations of seedlings did not change under more severe conditions of infestation, the data were pooled for statistical analysis. Expressing the results on the basis of the more severe infestation, White Martin had a mean of 55.9 aphids per plant, Piper 428-1-S1 had 19.2, the F_2 generation 31.1, and the F_3 generation 28.1 aphids per plant. The data for F_2 and F_3 generations had equal standard errors, and their means of infestation, though not statistically different, were closer to the mean of Piper 428-1-S1 than to the

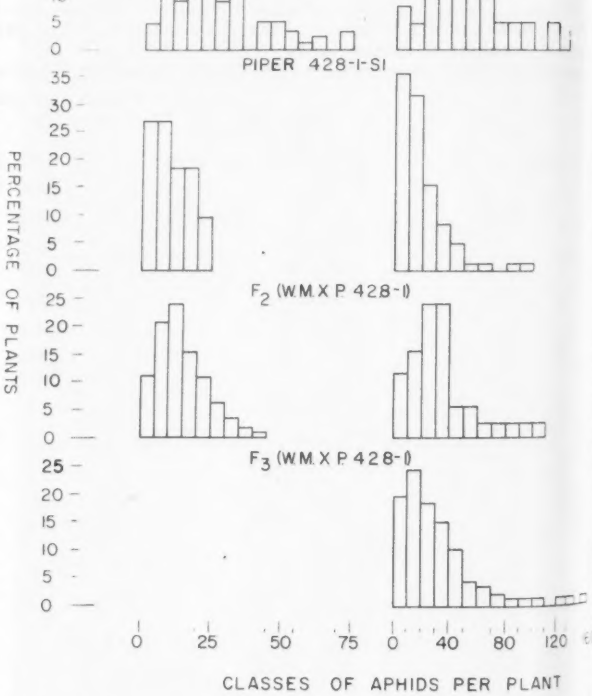

FIG. 3.—The results of two antibiosis screening tests of seedlings from the parental sources, and F_2 and the F_3 generations of the cross between White Martin and Piper 428-1, with a population of the biotype KS-1 of the corn leaf aphid. In the blocks on the left the classes were of 5 aphids per plant. On the right, the classes were of 10 aphids per plant. Manhattan, Kansas, 1955.

mean of White Martin. All the possible differences in infestation between White Martin, Piper 428-1-S1, the F_2 or the F_3 generations were significant. It should be noted that the Sudan grass plant 428-1 apparently was not homozygous for resistance to aphids, for a few fairly susceptible plants were found in its selfed progeny (Piper 428-1-S1).

In the second test, the F_3 generation was composed of 26 lines obtained from previous preference and antibiosis tests. The individual means of infestation of the 26 lines were compared with the preference or antibiosis readings of the F_2 parent plant. Generally the F_2 genotypes selected for non-preference produced F_3 lines with more antibiosis than other lines originating from more preferred F_2 selections. The F_2 genotypes selected for antibiosis produced F_3 lines with more antibiosis than other lines originating from F_2 genotypes carrying less antibiosis.

More complete and detailed antibiosis experiments were made on individual plants of the parental, the F_1, and the F_2 generations of the cross under investigation. The results, given in table 3, show that on White Martin the biotypes KS-1 and KS-2 had an equal rate of reproduction, but that the adults of KS-2 were heavier. On Piper 428-1, the fecundity of both biotypes was much reduced. Biotype KS-1 produced only one adult in four separate tests. Biotype KS-2 reproduced slowly and the adult was heavier than the one from biotype KS-1. On the F_1 hybrid, biotype KS-2 had a greater reproductive rate than KS-1 without any significant differences in the weight of the adults. Biotype KS-2 had the same rate of reproduction on the hybrid as on the susceptible parent, but the weight of the adults was reduced on the hybrid. In biotype KS-1, the reduction in fecundity of aphids reared on White Martin, F_1, and Piper was paralleled by a reduction in the weight of the adults. In biotype KS-2, feeding on the F_1 hybrid reduced the weight of the adults, did not reduce the fecundity; whereas feeding on Piper, in comparison with F_1, reduced the fecundity and did not reduce the weight of the adults. In figure 4, the effects of antibiosis on the weight and size of adults are illustrated with winged aphids reared on White Martin (susceptible) and Piper 428-1 (resistant).

In table 4 are shown the results of antibiosis cage tests with biotype KS-1 on two other crosses of sorghums

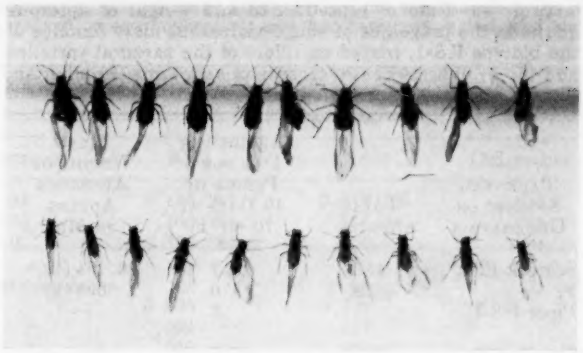

FIG. 4.—Effects of antibiosis on the weight and size of winged adults of the corn leaf aphid, *Rhopalosiphum maidis* (Fitch). Upper row: aphids reared on White Martin, a susceptible variety; the mean weight per aphid was 0.160 mg. Lower row: aphids reared on Piper Sudan grass 428-1, a resistant selection; the mean weight per aphid was 0.091 mg. Manhattan, Kansas. May 7, 1954.

that had Piper 428-1 as one of the parents. The antibiosis of Piper was transmitted to the F_1 generation. Along with the reduction in the fecundity of biotype KS-1 on the F_1 plants there was a sharp reduction in the weight of the adults produced. In these experiments no aphids matured on Piper 428-1.

The effects of antibiosis on the fecundity and weight of adults of aphids was followed in the F_2 generation with antibiosis cage tests. Limitations of technique, time, and space did not permit the simultaneous testing of more than 20 to 30 plants. In a first series of experiments, two sets of 12 plants each, selected at random for each biotype, were studied during a period of 18 days, with temperatures averaging 68.7° F. per day. As seen from the upper part of figure 5, the fecundity of the single females raised on those plants ranged from 0 to 11 aphids per day. Evidences of segregation of the plants resulting from differences in aphid fecundity were shown by grouping the results in classes of 0 to 1, 1 to 2, etc., aphids per day. The weight of the adults in the aphid progenies was positively correlated ($r=0.517^{**}$) with the increase or the reduction in the fecundity of the females reared on the plants.

In another series of studies on antibiosis in the F_2

Table 3.—Rate of reproduction and weight of apterous adults in the progenies of single viviparous apterous females of the biotype KS-1 and the biotype KS-2 of the corn leaf aphid, reared on the parents and the F_1 hybrid of the cross between White Martin and Piper 428-1. Manhattan, Kansas, 1955.

Host	Aphids per Day Periods of 14 to 23 Days			Weight of Apterous Adults in Mg.		
	KS-1	KS-2	Differences	KS-1	KS-2	Differences
A. White Martin	10.22 (3)[a]	9.04 (3)	1.18	0.166 (25)	0.207 (38)	0.041
B. F_1 Hybrid	2.31 (4)	8.43 (4)	6.12	.137 (10)	.130 (10)	.007
C. P. 428-1	.09 (4)	1.71 (3)	1.63	.082 (1)	.152 (14)	.070
			Differences between hosts			
A.–B.	7.91	0.61		0.029	0.077	
A.–C.	10.12	7.33		0.084	0.055	
B.–C.	2.22	6.72		0.055	0.022	

[a] Figures in parenthesis indicate the number of separate experiments under "aphids per day" and the number of individual aphids weighed elsewhere.

Table 4.—Rate of reproduction and weight of apterous adults in the progenies of single viviparous alate females of the biotype KS-1, reared on tillers of the parental varieties and the F_1 hybrids of two crosses of sorghums. Manhattan, Kansas, 1955.

Parental Source[a] or Generation	Total Aphids	Aphids per Day for a Period of 19 Days at 70–80° F.	Mean Weight of Apterous Adults in Mg.
Schrock-Ellis	451	23.7	0.204 (10)[b]
F_1	19	1.0	.094 (1)
Piper 428-1	5	.3	—
Combine 60	335	17.6	.200 (10)[b]
F_1 plant 2	27	1.4	.107 (2)
plant 4	11	.6	.064 (2)
plant 5	20	1.1	.119 (4)
plant 6	6	.3	.107 (2)
Piper 428-1	10	.5	—

[a] The plants were transplanted from the field to the greenhouse in October, 1954.
[b] A sample of the adults present. Number weighed given in parenthesis.

generation, the material was selected at random among a population of plants that had been screened previously for antibiosis and all of which had had an infestation of five or fewer aphids per plant. Two groups of 14 plants for each biotype were tested over a period of 19 days, with temperatures averaging 73° F. per day. As seen in the lower part of figure 5, the segregates producing lower aphid fecundity were considerably greater in number in this selected group, indicating the validity of the method of selection for antibiosis. The correlation between the increase in fecundity and the weight of the adults in the progenies of the females was positive and highly significant ($r=0.715^{**}$). In both series of antibiosis experiments, the apterous adults gave a higher correlation value than the alate adults.

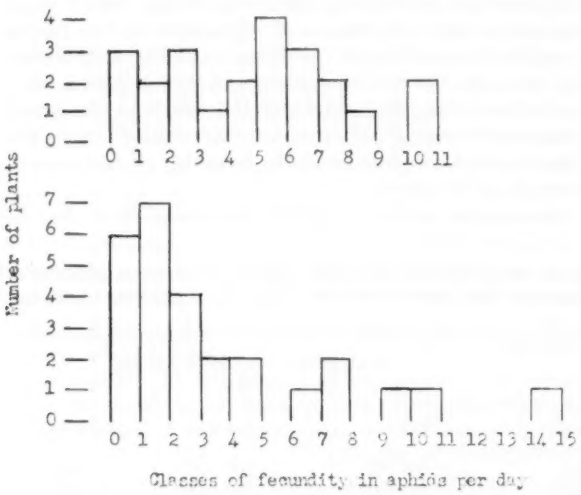

FIG. 5.—The segregation for antibiosis in F_2 plants of the cross between White Martin and Piper 428-1. The upper diagram was obtained with a non-selected group of 23 genotypes. The lower diagram was obtained with a group of 27 genotypes previously selected for antibiosis. The classes are of 0 to 1, 1 to 2, 2 to 3, etc., aphids per day. Manhattan, Kansas. 1955.

Because of the relatively small number of F_2 genotypes studied, it has not been possible to calculate a ratio of segregation of the plants in respect to antibiosis. The resistance and the susceptibility of the parents were recovered in F_2 with a rather gradual series of intermediate genotypes.

OTHER ASPECTS OF ANTIBIOSIS.—The antibiotic properties of Silver Top 1312 were studied along with White Martin and Piper 428-1-S1. Silver Top 1312 is a variety of sorghum which has been consistently of intermediate standing in preference tests. The results of antibiosis tests, as seen in table 5, indicated that Silver Top 1312 was also carrying antibiosis at an intermediate level. In this series of tests there were insufficient replications to give significance to the differences in fecundity in the biotypes. On Silver Top, biotype KS-2 had a higher fecundity and heavier adults than KS-1. On White Martin, biotype KS-1 had a higher fecundity but lighter adults than KS-2. On Piper 428-1-S1, the biotypes were practically equal in fecundity and weight. The effects of the susceptible, intermediate, and resistant hosts were a gradual reduction in the fecundity paralleled by a reduction in the weight of the adults.

The nature of antibiosis, as observed in the original selection Piper 428-1, was demonstrated as follows. Individuals of biotype KS-1 and KS-2 were reared simultaneously in separate cages on the same original plant. Their weight and reproduction were checked periodically. To accentuate the differences at the start, large individuals of biotype KS-1 and small individuals of biotype KS-2 were selected for the test. The results, as seen in table 6, showed that, while the individuals of biotype KS-1 lost weight and barely subsisted, the biotype KS-2 increased in weight and reproduced at the same time. Another series of tests with biotype KS-1 confirmed this phenomenon. Since it had been observed that, without food, the aphids died within 4 days, the length of the life of biotype KS-1 on Piper 428-1 was not much longer in some cases than it would have been without food.

The corn leaf aphid apparently develops very rapidly on the heads of corghums. Occasionally the aphids seemed much larger than usual. To investigate this problem, rearing experiments were started with biotype KS-1 and biotype KS-2, caging simultaneously and separately the head, the second leaf, and the fourth leaf from the top of maturing plants of White Martin. The results, as given in table 7, showed that the aphids reproduced two to five times faster on the heads than on the leaves. The weights of the apterous adults reared on the heads were double or triple those reared on the lower leaves of the same plants. Biotype KS-2 usually produced apterous adults significantly heavier than those produced by biotype KS-1.

During the analysis of the results of antibiosis, the ratio of apterous and alate aphids was observed to vary with the hosts and the biotypes. As seen in table 8, the biotypes also can be differentiated on the basis of the number of alate and apterous adults produced by the original apterous females when reared on White Martin, Piper 428-1, and their F_1 hybrid. On White Martin,

Table 5.—Rate of reproduction and weight of apterous adults in the progenies of single viviparous apertous females of the biotype KS-1 and the biotype KS-2 of the corn leaf aphid, reared on one susceptible and two resistant varieties of sorghums. Manhattan, Kansas, 1955.

Host	Aphids per Day Periods of 18 to 19 Days			Weight of Apterous Adults in Mg.		
	KS-1	KS-2	Differences	KS-1	KS-2	Differences
A. White Martin	11.03 (5)[a]	9.09 (5)	1.94	0.195 (39)	0.218 (53)	0.023
B. Silver Top 1312	3.43 (4)	5.37 (4)	1.94	.100 (26)	.136 (31)	.035
C. Piper 428-1-S1	2.43(4)	2.43 (4)	0.0	.102 (36)	.106(27)	.004
		Differences between hosts				
A.–B.	7.60	3.72		0.095	0.082	
A.–C.	8.60	6.66		.093	.112	
B.–C.	1.00	2.94		.002	.030	

[a] Figures given in parenthesis indicate the number of separate experiments under "aphids per day" and the number of individual aphids weighed elsewhere.

Silver Top 1312, and Piper Sudan 428-1-S1, there was very little difference in the number of apterous adults produced by the two biotypes, but the reduction of alate adults was considerable on the resistant hosts. Hence the biotypes can be differentiated on that basis also.

DISCUSSION.—The biology and the behavior of two biotypes of the corn leaf aphid have been analyzed quantitatively during preference and antibiosis tests in the presence of or on susceptible, intermediate resistant, and resistant host plants. Biotype KS-2 was able to reproduce itself on a resistant selection, Piper 428-1, while biotype KS-1 only occasionally barely subsisted. Under several conditions of experimentation, the biotype KS-2 always produced heavier adults than biotype KS-1. These results in part, were similar to those obtained by Harrington (1943) with the pea aphid. Harrington found that high survival capacity was apparently associated with added size. More recently, Harrington (1945) tentatively grouped his results of extensive work on 31 lines of pea aphids into five biological races of progressive virulence. Our results have shown, however, that not only the biotypes of the insect may be responsible for the added size but that, on the same host, the reactions of two biotypes may be differentiated in terms of fecundity, weight of adults, and number of alate adults in the progenies of apterous females. The various features by which the two biotypes may be distinguished are summarized in table 9.

Table 6.—Rate of reproduction and measurements of weight of apterous females of biotype KS-1 and biotype KS-2 of the corn leaf aphid, during their lifetime on Piper 428-1. Manhattan, Kansas, November 5 to December 7, 1954.

Biotype	Weight of the Original Females[a] (Mg.)				Total Progeny	Aphids per Day	Mean Weight of Apterous Adults in the Progeny (Mg.)
	Nov. 5	*Nov. 10*	*Nov. 15*	*Nov. 21*			
KS-1	0.484	0.260	dead		1	0.06	—
	0.472	0.276	0.240	dead	1	.06	—
KS-2	0.110	0.120	0.128	0.100	46	2.88	0.179 (5)[b]
	0.110	0.138	0.138	0.080	58	3.62	.125 (6)
	Nov. 21	*Nov. 23*	*Nov. 29*	*Dec. 7*			
KS-1	0.490	0.270	0.176	dead	8[c]		
	0.418	0.270	lost		0		
	0.362	0.252	0.154	dead	0		
	0.476	0.246	dead		0		

[a] All the individuals from another group of females caged without food died within 4 days.
[b] Number of measurements given in parenthesis.
[c] On December 21, this colony was still alive but increasing very slowly.

The resistance of sorghums to the corn leaf aphid, as observed in previous studies, may be the result of three basic components described by Painter (1941) as preference, antibiosis, and tolerance. In Howitt's (1954) and in present studies, preference and antibiosis only were analyzed in their effects on the biology of the aphid.

The results have shown that there exist, in sorghum varieties and hybrids, some qualities which remained

Table 7.—Rate of reproduction and measurements of weight of biotype KS-1 and biotype KS-2 of the corn leaf aphid, when reared simultaneously and separately on the heads and the lower leaves of maturing White Martin sorghums. Manhattan, Kansas, 1955.

Position of Cages	Aphids per Day for a Period of 13 Days			Weight of Apterous Adults (Mg.)		
	KS-1	KS-2	Differences	KS-1	KS-2	Differences
On the head	15.69	23.46	7.77	0.337 (24)[a]	0.471 (20)	0.134
	10.08	21.23	11.15	.348 (21)	.385 (19)	0.035
On the 2nd leaf from the top	1.08	5.46	4.38	.139 (2)	.250 (10)	.111
	2.69	9.31	6.62	.140 (6)	.174 (19)	.034
On the 4th leaf from the top	7.23	3.08	4.15	.195 (15)	.263 (12)	.068
	4.31	9.08	4.77	.132 (9)	.178 (18)	.046

[a] Numbers weighed from each replicate given in parenthesis.

Table 8.—Relation of antibiosis to the production of alate and apterous adults in the progenies of single viviparous apterous females of biotype KS-1 and biotype KS-2 of the corn leaf aphid. Manhattan, Kansas, 1955.

Form of Adults Produced and Biotype		Total Number of Adults Obtained from the Antibiosis Cage Tests			Chi-Square Tests to Differentiate the Biotypes
		White Martin	*F_1 hybrid*	*Piper 428-1*	
Apterous	KS-1	41 (5)[a]	11 (4)	1 (4)	Significant
	KS-2	53 (5)	36 (4)	13 (4)	
Alate	KS-1	84 (5)	6 (4)	0 (4)	Significant
	KS-2	45 (5)	52 (4)	6 (4)	
		White Martin	*Silver Top 1312*	*Piper 428-1-S1*	
Apterous	KS-1	24 (3)	26 (4)	36 (4)	Not significant
	KS-2	24 (3)	31 (4)	27 (4)	
Alate	KS-1	57 (3)	7 (4)	0 (4)	Significant
	KS-2	34 (3)	23 (4)	4 (4)	

[a] Number of separate tests conducted are indicated in parenthesis.

constant under varying intensities of infestations. In populations segregating for preference and non-preference the preference of the F_2 generation was intermediate between the parents, as shown in table 2. The inheritance of non-preference was demonstrated in plastic box preference tests, as seen in table 1. In the cross White Martin×Piper 428-1, the non-preference of Piper (a component of resistance) was transmitted as a dominant character. The alate of two biotypes of the corn leaf aphid responded by a same pattern of distribution over leaf cuttings of these hosts. In this reaction, two to three times as many alate aphids preferred White Martin to either the F_1 hybrid or Piper 428-1. The females also gave birth to more nymphs on leaf cuttings of White Martin than on the other leaf cuttings, in less than 1 hour. The pattern of distribution of the adults and the distribution of the nymphs born during the test hour may be the result of two separate factors.

Antibiosis was measured on the parental, the F_1, the F_2, and the F_3 generations of the cross between White Martin and Piper 428-1. In testing populations of these generations, the intensity of the infestation was calculated on the basis of number of aphids produced per parent aphid per plant. The variation about the mean was analyzed and the results presented in the form of histograms, as seen in figure 3. White Martin was represented by the variety itself and Piper 428-1 by its progeny. The dominance of antibiosis was seen in the fact that the means of infestation in the F_2 and F_3 populations were closer to the mean of the resistant parent than to that of the susceptible parent.

More complete and detailed antibiosis experiments were undertaken with the rearing of aphids in cage tests on White Martin, Piper 428-1, and their F_1 hybrid. As seen in table 3, the expression of antibiosis in reducing the fecundity of biotype KS-1 behaved as a completely dominant factor in Piper, partially dominant in the F_1, and recessive in White Martin; in reducing the weight of the adults, the character appeared to be expressed as a dominant in Piper, partially dominant in the F_1, and recessive in White Martin. The expression of antibiosis in reducing the fecundity of biotype KS-2 appeared to be dominant in Piper 428-1 and recessive in the F_1 and White Martin; while, in reducing the weight of the adults, the expression appeared to be partially dominant in Piper 428-1 and the F_1, and recessive in White Martin. There was a segregation for ability of the plants to suppress fecundity and reduce weight in the F_2 generation. The resistant genotypes reduced the fecundity and the weight of the adults in the progenies of apterous females. The susceptible genotypes favored a rapid rate of reproduction and increased the weight of the adults.

The nature of antibiosis of the original selection was demonstrated by the results presented in table 6. The females of biotype KS-1 lost weight as soon as they fed on that host, and their progeny did not mature. The females of biotype KS-2 were affected to a lesser degree. In Piper 428-1-S1, the near immunity of the parent against biotype KS-1 had been lost (Table 5). Biotype KS-1 was not more affected than KS-2 on that host. The antibiosis of the original plant selection must have been the result of a peculiar genic composition.

In considering other aspects of antibiosis, it was shown by the results presented in table 5 that Silver Top 1312, which was of intermediate standing in preference tests, also carried antibiosis at an intermediate level. It appears that preference and antibiosis were related genetically or physiogically. Antibiosis inhibited totally or partially the mechanisms of wing production in the progenies of apterous females. This relation of antibiosis and reduction of winged adults appeared to demonstrate that there existed in the plants some factors that influenced the mechanisms of wing formation. The use of resistant varieties of sorghums might lower the population of winged adults and affect, in part, their natural migration.

On the basis of the evidence presented on preference and antibiosis, the total resistance of sorghums (not including tolerance) would be the result of several or all the following mechanisms:

1. Lack of attraction for winged aphids or repellence either from a distance or after probing.
2. Lack or inhibition of stimulus for birth of nymphs.
3. Reduction in number of nymphs born.
4. Lack or inhibition of stimulus for feeding.
5. Reduction in the weight of adults produced.
6. Total or partial inhibition of wing formation in the progenies of apterous females.

Table 9.—Some features of the biotype KS-1 and the biotype KS-2 of the corn leaf aphid, when reared on some varieties of sorghums. Manhattan, Kansas, 1955.

Host Plant	Biotype KS-1	Biotype KS-2
White Martin	1. High fecundity[a] 2. Heavy adults	1. High fecundity 2. Heavier adults
Piper 428-1	1. Very low fecundity 2. Very light adults 3. Large alate losses weight 4. Restlessness	1. Low fecundity 2. Medium weight adults 3. Small alate gains weight 4. No restlessness
F_1 hybrid W.M.×P. 428-1	1. Low fecundity 2. Medium weight adults 3. Some mortality 4. Restlessness 5. Population aggregates in the whorl	1. Medium fecundity 2. Medium weight adults 3. Less mortality 4. No restlessness 5. Population aggregates along the stem
Silver Top 1312	1. Low fecundity 2. Medium weight adults	1. Low fecundity 2. Heavier adults

[a] The trends given for fecundity and weight of adults correspond to statistically significant differences.

Some of these mechanisms may be related, such as Nos. 2 and 3. It was not shown whether No. 5 would be a consequence of No. 4. The factors responsible for these mechanisms appeared to be either dominant or partially dominant in their expression because the aphid biotypes reacted differently to the same resistant host plants. The non-discrete segregation in the F_2 plants and the F_3 lines suggests a multiple factor hypothesis.

The possibility of developing corn leaf aphid resistant combine-types of sorghums appears to be promising. Not only would there be a reduction in the damage done to the resistant sorghums by aphids, but there should also be a considerable reduction in the population of aphids in a field of resistant sorghums.

SUMMARY AND CONCLUSIONS.—Two biological races of corn leaf aphid were discovered and classified as biotype KS-1 and biotype KS-2. Biotype KS-2 had a greater survival capacity on resistant varieties, and under comparable conditions produced heavier adults than biotype KS-1. These biotypes showed a number of other differences.

The resistance of sorghums to the corn leaf aphid was analyzed by measuring separately the mechanisms of preference and antibiosis. Tolerance may be an important component of resistance as seen in the field, but so far has been difficult to study.

In measuring preference in large groups of plants, the basis of comparison was the number of infestations per plant. In leaf cuttings of individual plants mounted in plastic boxes, the pattern of distribution of winged aphids on leaf cuttings after 1 hour was the measure of the preference qualities of the plants.

In measuring antibiosis in large groups of plants, the basis of comparison was the actual number of aphids produced per plant during a given period, following a repeated initial infestation of each plant. On individual plants, antibiosis was measured by the main increase (aphids per day) of the progeny of single viviparous females caged on the plants.

In 50 varieties, hybrids, and selections of sorghums, involving 5,000 plants, preference was a part of the inherited qualities that remained relatively constant under varying intensities of infestations.

Non-preference, as observed in the F_1 hybrid of a cross between White Martin (preferred) and Piper 428-1 (non-preferred) behaved like a dominant character. The average for F_2 generation was intermediate between the parents in reaction to aphids.

There were in White Martin (preferred) some factors which, within a test period of *1 hour*, attracted and stimulated the females to give birth to nymphs. Conversely, there were in Piper 428-1 (non-preferred) some factors which, within a test period of 1 *hour*, repelled and almost totally prevented the females from giving birth to nymphs.

The antibiosis of Piper 428-1 behaved as a dominant character in F_2 and F_3 populations.

Non-preference in F_2 genotypes and standard varieties appeared to be related to antibiosis either physiologically or genetically, or both.

The expression of antibiosis in reducing the fecundity of biotype KS-1 behaved as a complete dominant in Piper 428-1, partially dominant in the F_1, and recessive in White Martin; in reducing the weight of the adults, the character appeared to be expressed as dominant in Piper 428-1, partially dominant in the F_1, and recessive in White Martin.

The expression of antibiosis in reducing the fecundity of biotype KS-2 appeared to be partially dominant in Piper 428-1 and recessive in the F_1 and White Martin; while, in reducing the weight of the adults, the expression appeared to be partially dominant in Piper 428-1 and F_1, and recessive in White Martin.

The antibiosis of Piper 428-1 affected biotype KS-1 by reducing the weight of the females as soon as they fed on that host and preventing the maturing of their progeny, if any. Biotype KS-2 was affected to a lesser degree. In the progeny of Piper 428-1, biotype KS-1 was not less affected than biotype KS-2.

There was a segregation for ability of the plants to suppress fecundity and reduce aphid weight in the F_2 generation. The antibiosis of the resistant parent and the susceptibility of the other parent were recovered in several F_2 plants. As observed from the progenies of single viviparous females caged on the plants, the resistant genotypes reduced the fecundity of the females and the weight of the adults in the aphid progenies. The susceptible genotypes favored a rapid rate of reproduction and increased the weight of the adults in the aphid progenies.

Aphids of both biotypes reared separately on the heads of White Martin were heavier and their rate of reproduction was double or triple that of others reared on lower leaves of the same plants.

Antibiosis at least partially inhibited the mechanisms of wing production in the progenies of apterous females.

Analysis of resistance in the sorghums studied indicated that six mechanisms of either preference or antibiosis were responsible for the differences found. These mechanisms appeared to be either dominant or partially dominant in their expression because the biotypes showed differential reactions on the same host plants. The non-discrete segregation in the F_2 plants and the F_3 lines suggests a multiple factor hypothesis.

REFERENCES CITED

Barnhart, C. S., and L. E. Chadwick. 1953. A "fly factor" in attractant studies. Science **117:** 104–5.

Harrington, C. D. 1943. The occurrence of physiological races of the pea aphid. Jour. Econ. Ent. **36:** 118–9.

Harrington, C. D. 1945. Biological races of the pea aphid. Jour. Econ. Ent. **38:** 12–22.

Howitt, A. J., and Reginald H. Painter. 1956. Field and greenhouse studies regarding the sources and nature of sorghum (*Sorghum vulgare* Pers.) resistance to the corn leaf aphid, *Rhopalosiphum maidis* (Fitch). Kansas Agric. Expt. Sta. Bull. **82.**

Huber, L. L., and G. H. Stringfield. 1942. Aphid infestation of strains of corn as an index of their susceptibility to corn borer attack. Jour. Agric. Res. **64:** 283–91.

McColloch, J. W. 1921. The corn leaf aphis (*Aphis maidis* Fitch) in Kansas. Jour. Econ. Ent. **14:** 89–94.

Painter, R. H. 1941. The economic value and biological significance of plant resistance to insect attack. Jour. Econ. Ent. **34:** 358–67.

Painter, R. H. 1951. Insect resistance in crop plants. New York: The Macmillan Co. 520 p.

Smith, H. S. 1941. Racial segregation in insect populations and its significance in applied entomology. Jour. Econ. Ent. **34**: 1–13.

Snelling, R. O., R. A. Blanchard, and J. H. Bigger. 1940. Resistance of corn strains to the leaf aphid, *Aphis maidis* Fitch. Amer. Soc. Agron. Jour. **32**: 371–81. 31 ref.

Thorpe, W. H. 1930. Biological races in insects and allied groups. Biol. Rev. **5**: 177–212.

Thorpe, W. H. 1940. Ecology and the future of systematics, *in* J. S. Huxley, "The new systematics." New York: Oxford Univ. Press. 341–64.

Viale, E. 1950. The biology of the corn leaf aphid, *Aphis maidis* Fitch, as affected by various strains of corn, *Zea mays* L., and certain other environmental factors. Unpublished Ph. D. thesis, Kansas State College, Manhattan, Kansas.

Walter, E. V., and A. M. Brunson. 1940. Differential susceptibility of corn hybrids to *Aphis maidis*. Jour. Econ. Ent. **33**: 623–8.

Wildermuth, V. L., and E. V. Walter. 1932. Biology and control of the corn leaf aphid with special reference to the southwestern states. U. S. Dept. Agric. Tech. Bull. **306.**

Report on the Khapra Beetle Program in Arizona[1]

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With the possible exception of the pink bollworm, *Pectinophora gossypiella* (Saund.), no other insect pest or plant disease has had such a disastrous impact upon the economy of the State of Arizona as the khapra beetle, *Trogoderma granarium* (Everts). Actual losses of products destroyed by this insect have been relatively small, probably not over $10,000.00; however, incidental losses resulting from control measures, commodity treatments to comply with quarantines and eradication treatments together with loss of business due to a multiplicity of conflicting state quarantines, unfair trade discrimination and ill-advised regulations made by the Commodity Credit Corporation, have resulted in staggering losses.

Since State and Federal Quarantines were promulgated it has cost the grain industry of Arizona approximately $20,000.00 a month for commodity treatments to comply with quarantines. Control measures exclusive of sanitation programs have cost in excess of $100,000.00 and much of this money was a total loss due to ineffective measures used. Before the advent of the khapra beetle, Arizona had an annual million dollar trade in certified sorghum and barley seed. A large percentage of this trade has been lost due to the fact that processing houses were infested and seed fumigation was required. Methyl bromide fumigation lowers seed germination under certain circumstances. One firms claims over $4,000.00 in adjustments due to lowered seed germination.

Losses due to unfair trade practices cannot be estimated but for many months the Los Angeles Grain Exchange quoted prices only on grain from non-infested establishments. The Commodity Credit Corporation refused storage contracts on any establishments listed as infested under the Federal Quarantine. The loss to Arizona from this source was by no means small. In addition to all other losses and costs to Arizona, $119,190.52 has been spent in the co-operative eradication program, the details of which are described in this paper.

HISTORY OF KHAPRA BEETLE IN ARIZONA.—Khapra beetle was first identified in Arizona on January 21, 1954, although there is evidence that it was present in considerable numbers as early as July 1953. The "Cooperative Economic Insect Report" issued December 18, 1953, by the U. S. Department of Agriculture reported a dermestid in grain storages in Phoenix. An immediate investigation was started and in late December specimens collected by inspectors of the Department of Agriculture and the State of Arizona were sent to Washington for identification, the report, of which was received on January 21. As soon as it was definitely known that khapra beetle occurred in Arizona a delimiting survey was started. This has been a continuing survey from that time on and, to date, 78 properties have been found infested out of 1800 inspected.

CONTROL.—The initial control measure recommended by the State Entomologist's Office was absolute sanitation. A spray containing 0.5% lindane in dormant oil was tested on a large, flat storage bin with laminated jack walls. These walls were saturated three times with lindane spray under 400 lbs. pressure. Observations indicated that a good kill resulted and new crop barley was stored in the same storage. There was apparently sufficient survival in the cracks between the boards of the jack wall so that in 5 months the new crop barley was heavily infested. Wetable 50% DDT at the rate of 40 pounds was also tested on building walls and although fair control was obtained neither the penetration nor the residual effect was sufficient to warrant its recommendation. All early attempts at control were of doubtful value.

QUARANTINES.—As soon as the delimiting survey had progressed to the point that it seemed practical to do so, the State of Arizona began formulating a plan for regulatory measures to prevent the further spread of this insect. Early attempts at regulation were most discouraging. This was the first time on record that an attempt had been made to regulate a strictly storage pest. With the assistance of Mr. L. J. Padget, who is in charge of the Khapra Beetle Project for the U. S. Department of agriculture, an intrastate quarantine was written and filed with the Secretary of State for a waiting period and public hearing as required by Arizona Law. The hearing was held on September 27, 1954 and was attended by many representatives of the grain and seed industry. As a result of legal questions which were raised the Arizona

[1] Presented at the Third Annual Meeting of the Entomological Society of America at Cincinnati, Ohio, November 28, 1955. Accepted for publication February 6, 1956.

Commission postponed action on an intrastate quarantine. The failure to enact a quarantine touched off a chain reaction of separate State Quarantines. Colorado placed an embargo type quarantine against Arizona and California and this was followed by 17 different state quarantines. These quarantines did serve a purpose; they focused the attention of the entire country on this problem and started action to secure Federal assistance and co-ordination.

After another unsuccessful attempt the State of Arizona finally adopted an interior quarantine on December 29, 1954. This was a compromise regulation and under its provisions the treatment of all commodities moving from an infested property was required except that, under special permit, materials could be moved for immediate planting or feeding without treatment. The entire county was placed in a regulated status and permits for movement outside the county were required from noninfested as well as infested properties. Under the terms of this quarantine the State could force the property owners to employ control measures.

Through the co-operation of the Stored-Product Insects Section of the Agricultural Marketing Service, U. S. Department of Agriculture, a schedule of treatments had been developed permitting safe movement of infested products without danger of further spread. Some of the treatment schedules have since proved to be insufficient but the majority of them were more than adequate.

As a result of a Hearing which was held in Denver, Colorado, on December 1, 1954, the U. S. Department of Agriculture drafted Federal Domestic Quarantine No. 76 which became effective February 28, 1955. The provisions of this quarantine were similar to the Arizona Quarantine except that only the infested properties were declared the regulated area. Treatment schedules for movement of commodities were the same as required by the Arizona and California quarantines.

RESEARCH.—In solving the problems of the khapra beetle the regulatory agencies were extremely fortunate in having the prompt and whole-hearted support of research agencies. The Stored-Product Insects Section of the Agricultural Marketing Service, worked out early treatment schedules from data at hand and immediately established research projects in Arizona and California. The University of California and the University of Arizona set up projects at a very early date. Agricultural Research Services as well as commercial firms have contributed to the research effort.

The Stored-Product Insects Research Project in Arizona was originally set up to work on commodity treatments; however, considerable work was done in 1954 on control measures. One large scale fumigation using a liquid fumigant was conducted. In this test a liquid fumigant of ethylene dibromide, ethylene dichloride and carbon tetrachloride was used at a rate of 3½ gallons to each 1,000 bushels of barley. In this test 1,100 gallons of fumigant were used and an estimated kill of over 90% was obtained; however, there was some survival and after the grain had been fed the storage was torn down and rebuilt. This was, perhaps, the most successful test with a liquid fumigant. Others gave very doubtful results and the liquid fumigants were abandoned as control measures.

In California various insecticides were tested for their lethal and residual qualities and some tests were conducted in Arizona. Malathion was finally adopted as the most promising material, although, it must be admitted it is not entirely satisfactory due to its limited residual effect.

Sanitation still proved to be the most effective control with the exception of methyl bromide fumigation which was adopted as the standard treatment for commodities to be moved under quarantine. Heat treatments were tested and later heating to 180° F. was adopted as an alternate commodity treatment.

Although none of the methods tested proved entirely satisfactory as a control yet a combination of all methods rigidly enforced by regulatory inspectors has proved so effective that it is difficult to find khapra beetle in Arizona at the present time. Only in one place in the State is there a heavy infestation. This particular storage was fumigated approximately 9 months ago with a liquid fumigant into which had been bubbled 8 ounces of methyl bromide per gallon and a fair kill obtained. However, the nature of the storage was such that the fumigant could not be contained and the test was not considered successful.

ERADICATION TESTS.—From the outset regulatory officials have pointed their program towards a complete eradication of the khapra beetle from the United States. During the early days of the problem it looked hopeless. The Pest Control Branch of the U. S. Department of Agriculture was optimistic from the beginning and, although they recognized that it would take time and be very expensive, they felt that it could be accomplished. Mr. H. M. Armitage, with his store of knowledge and experience and backed by California with its unlimited finances, was ready and willing to undertake tests to determine whether or not the khapra beetle could be eradicated from a given premise. A report on these tests appears in the preceding paper.

Arizona's start on this project, which has been termed the largest fumigation job in the world, was less portentous than California's tests. With limited funds appropriated by the Legislature, plastic tarpaulins, clamps and a Gow-Mac thermo-conductivity gas analyzer were purchased. With spray equipment already owned by the State, Arizona went to work on small properties and farm storages, doing most of the work and getting what contributions could be supplied by the property owners. By this procedure we were able to give our inexperienced personnel some training before getting into the major program.

ERADICATION PROCEDURES.—In early April 1954, at Williams, California, the results of the research and the fumigation tests were analyzed by the research experts and the regulatory officials of the U. S. Department of Agriculture and the states involved and the pattern for the Federal-State co-operative program for the eradication of the khapra beetle was evolved. To briefly review this program, an infested property would be eligible for release from quarantine if all of the buildings were completely covered with a gas impervious material and fumigated as follows:

(1) The initial application of methyl bromide was set at 5 pounds per 1,000 cubic feet of space under the cover. The gas tight seal was to be maintained for a period of 48 hours. The concentration of gas was to be sampled at 2, 4, 6, 12, 18, 24, 36 and 48 hours. Gas sampling points

were to be established in critical locations throughout the buildings and the commodities within the buildings and a concentration of not less than 32 ounces per 1,000 cubic feet was to be maintained for 24 consecutive hours out of the 48-hour period. This concentration and time was believed to be a safety factor of at least 2½ over the lethal dosage for the insect.

(2) When bulk commodities were included a forced circulation system would be installed which would draw the air from the bottom of the mass and hasten the penetration of the fumigant.

(3) The environs are thoroughly cleaned before fumigation and the area sprayed with a spray of 5 lbs. of actual malathion to 100 gallons of diesel oil, or water emulsion where oil cannot be used. Spraying is done before, during, and after fumigation and the ground stirred between sprayings.

As soon as possible after the meeting at Williams the program was set in motion in Arizona. The U. S. Department of Agriculture agreed to furnish the fumigant and the technical supervision which would normally be about one-third of the cost. The State of Arizona was not financially able to pay for the balance of the cost as was done in California but with the help of the Plant Pest Control Branch of the U. S. Department of Agriculture, an agreement was reached with the grain and seed dealers' organization whereby the State would assume the cost of spraying and application of the fumigant provided the property owner would assume the cost of covering the buildings.

PROGRESS IN ARIZONA.—Between May 28 and November 8, eradication type fumigations have been applied to 30 properties. Fourteen were farm and ranch properties and 16 were commercial type establishments. Approximately 10,800,000 cubic feet of warehouse space has been fumigated out of an estimated 30 million cubic feet which was known to be infested. These properties range in size from a small feed store containing 5,100 cubic feet to a mill and elevators of 2,600,000 cubic feet.

To give some idea of the magnitude of one of these operations, the largest warehouse fumigated in Arizona, the 2,600,000-cubic-foot job, required approximately 8 acres of nylon plastic tarpaulin to cover the buildings and attachments. These tarpaulins cost approximately $60,000.00. To join these tarpaulins together required about 27,000 clamps. Over 6½ tons of methyl bromide was applied to this building. To introduce this methyl bromide into the proper location required 40 introduction lines and into these lines went 40 pressure gages, 100 ft. of copper tubing, 483 ft. of ½-inch black pipe, 200 ft. of high pressure neoprene tubing, 3,650 ft. of polyethylene tubing.

To circulate the fumigant into bulk commodities required 55 commercial type vacuum cleaner motors, forty-four 1¼-inch well points or sand points, 666 ft. of 1¼-inch pipe and 4,000 ft. of electrical wiring together with control panels and switches to operate these motors. To circulate the fumigant in the open spaces of the buildings requires seven 4,500-cubic-feet-per-minute circulation fans and six floor fans. The treatment of the grounds and environs required the application of 52 gallons of 25% malathion emulsion and 1,935 gallons of diesel oil. The cost of applying the environs treatment was $470.00.

To test the results of the fumigation required one house trailer equipped as a mobile laboratory. Two Gow-Mac thermoconductivity gas analyzers were used. Seventy-five test stations were established which required 25,000 ft. of ¼-inch plastic tubing and 50 special stainless steel probes for sampling in bulk and sacked commodities. It required 544 man hours of labor exclusive of supervision to install the circulation and testing equipment. Six technical supervisors were on the job most of the time during a 2-week period of preparation and fumigation. The owners of the plant moved to another location for operation during a week's period and paid $12,600.00 on a contract for covering the building.

SPECIAL PROBLEMS.—Many special problems were encountered in developing and conducting this eradication program. Trained personnel was the first problem. When this program started there was no one in the United States with experience in conducting fumigations of this magnitude. The Pink Bollworm Project of the U. S. Department of Agriculture furnished one experienced man who made great contributions. The experience of a few commercial exterminators who had performed house fumigations was utilized to the fullest. The manufacturers of the Gow-Mac Instruments gave valuable aid and trained instrument operators.

In the main the men who are now considered as experts on this work were trained on the job. With the combined knowledge and experience of the men working on the project, the size of a fumigation job is now limited only by the funds available.

The next big problem was the special equipment which was necessary. Most of the equipment was designed and fabricated on a trial and error basis without the assistance of trained engineers. Tarpaulins had to be made to order and commercial type clamps were adapted to fit the problem of joining them together. Circulation systems were made up of vacuum cleaner blowers and obsolete well points. Stainless steel sampling probes were made up to order. Fans and blowers of all types were adapted to fit the needs. Much labor and material was wasted but it was an emergency which amply justified some waste.

Technical difficulties were numerous. As an example, research workers found that the sorption qualities of host material varied greatly and that with the gas concentration used on some products there was little or no penetration. Finely ground meals with high fat and protein contents absorbed the gas to the extent that with 5 pounds of methyl bromide per 1,000 cubic feet there was little penetration. It was determined that it took 12 pounds to get sufficient concentration into sacked cotton seed meal. This made it necessary to do a fumigation within a fumigation when cotton seed meal was stored in a warehouse being treated. The meal products were stacked in a separate pile, covered with a tarpaulin and a special injection line run to this stack so that 12 pounds could be injected.

The extreme heat during the summer months caused the gas analyzer to malfunction and numerous repairs were necessary. Erratic readings caused many delays and required extra fumigations.

Weather was a vital factor in this work. Temperatures of 118 degrees F. made it difficult to keep laborers. Work on metal buildings had to be done at night. High humidity caused condensation in sampling lines and erratic readings on the analyzer. Wind was the most disturbing factor.

Thousands of dollars worth of nylon could be torn to bits in a few minutes. During the period of summer thunderstorms the cover was torn off seven times before one job was finished and it took almost a month to complete it.

COST OF FUMIGATION.—The following are the approximate figures on the cost of the eradication type treatments applied to the 30 infested properties in Arizona

A total of $119,190.52 was expended from funds set aside for this project. This does not include the salaries and expenses of personnel of the U. S. Department of Agriculture and the State of Arizona who are on a permanent status and who plan and supervise the fumigations. By agreement, their salaries and expenses are not charged to the eradication project. With this item added it is estimated the costs would be increased by $25,000.00.

The basis for planning and computing costs of these fumigations is the 1,000-cubic-foot unit. The 30 fumigations in Arizona have cost an average of $11.00 per 1,000 cubic feet. This is considerably below the original estimate which was set at $17.00. Due to the diverse conditions encountered in treating farm storages the cost of the 14 farm storage units treated in Arizona averaged $14.07 per 1,000 cubic feet.

On those properties where the covering of the buildings was done by Federal and State employees, with Federal and State equipment, the cost per 1,000-cubic-foot unit was $13.44 as compared to a unit cost of $10.63 where the covering of the buildings was done under commercial contract. The lowest per unit cost on any one job was $6.39; the highest, $48.58. On properties of over a million cubic feet, the average cost was $10.84. Generally speaking, large warehouses cost less per 1,000-cubic-foot unit than do small establishments of less than 100,000 cubic feet.

Under the system of financing employed in Arizona the property owners have contributed approximately one-half the direct costs and the Federal and State agencies have divided the other one-half. Were the costs of planning and supervision added the conventional one-third formula would more nearly apply.

CONCLUSIONS.—The khapra beetle has cost the State of Arizona well over a million dollars since January 1954. This loss is small compared to the potential destructiveness of this insect. The money expended in eradication and control work has provided good insurance against greater losses.

Through the splendid co-operation of the property owners and the Federal agencies involved, a fair degree of control has been achieved and actual losses reduced to a minimum. Through the medium of Federal and State quarantines together with control measures, the uninfested portion of the United States is now being protected against infestation. The degree of protection is at least as good as that afforded by any other domestic quarantine.

As a result of the techniques which have been developed it is entirely within the realm of possibility that the khapra beetle can be eradicated from the United States. Eradication will be a long and difficult process and it will require at least 2 more years to definitely determine the possibility. Considerably more money is needed to complete the eradication program and a larger percentage of the cost must be borne by the Federal and State governments in order to complete the task.

Toxicity of Pesticides to *Typhlodromus fallacis* (Gar.)[1]

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Many excellent papers have been published illustrating the influence of new pesticides and pesticide combinations on the beneficial orchard fauna, especially predaceous mites. Lord (1948, 1949) pointed out the harmful effect of DDT, sulfur and ferbam to predatory mites. Cutright (1944) also showed the deleterious effect of sulfur and ferbam to predaceous mites. Clancy (1952, 1953, 1954), Pickett (1953), MacPhee (1954) and Stevenson (1955) also presented evidence that some pesticides and pesticide combinations are more toxic to beneficial arthropods than others. Huffaker (1953) demonstrated that some predatory mites, i.e. *Typhlodromus occidentalis* Nes., are surprisingly resistant to parathion.

All of these papers, however, report on field experiments. Admittedly the field is the final judgment ground. However, an experimenter can answer many of the preliminary problems in the laboratory. He can save time, energy and money through carefully organized and properly interpreted laboratory tests. With this premise in mind, we began a search for techniques to evaluate the toxicity and influence of pesticides to the eggs and motile forms of the predaceous mite, *Typhlodromus fallacis*.

METHODS AND MATERIALS.—The toxicity of chemicals can be measured in several ways, (1) initial toxicity to the motile stages, (2) residual toxicity to the motile forms. (3) ovicidal action. Other characteristics of the chemical, such as repellency and inhibition of oviposition, may also be important.

The previous paper (Ristich 1956) mentioned the fact that testing techniques are not easily designed to measure the influence of chemicals on the small, but swift, adult stage of this phytoseiid mite. Although contact and residual data can be obtained if leaves, with the mites, are sprayed and placed in the two-unit plastic cups, more precise and accurate information is obtained if the motile forms are first killed with TEPP. Both the two-unit plastic cup method and the TEPP method (at 50 p.p.m.) which kills the motile forms, were used to gather most of the data for this paper. The information in tables 1 to 3 was recorded by the leaf-dip method described by Daugherty (1953).

In most situations no attempt was made to measure the toxicity of pure chemicals. Some proprietary chemicals, such as Dieldrex 15 EM,[3] or benzal azine[4] 20 EM, are four

[1] Accepted for publication January 13, 1956.

[2] Present address, Olin Matheison Chemical Corporation, c/o John Powell Laboratory, Port Jefferson, N. Y.

[3] A 15% dieldrin emulsion.

[4] $C_6H_5CH{=}NN{=}CHC_6H_5$.

times as toxic as the technical grade preparation. Here the solvent, in a five or six to one ratio, is more toxic than the chemical. Presumably where more critical comparisons are needed, as in tables 7 and 8, dieldrin and DDT were formulated with the same solvent and emulsifier. The situation is different but the conditions are nevertheless arbitrary. Regardless of the formulation, however, all dosages are expressed as actual percentage of the active ingredient. All the tests were replicated at least twice and all the follow-up tests were replicated three or four times.

The primary premises of the paper are, (1) to separate the pesticides into graded toxicity groups, (2) to compare a few related analogs, (3) to investigate the mode of action of ferbam and sulfur and, (4) to investigate the influence of a pretreatment of one chemical on the toxicity of another superimposed shortly afterward. Perhaps other entomologists will someday devise more accurate testing methods and eventually solve some of the more fundamental problems.

RESULTS.—The data in table 1 represent 24 toxicity ratings of fungicides, acaricides and insecticides, against the motile stages. The phosphorous-type insecticides, parathion and malathion, DDT and its analog DMC were the most toxic. Chlorobenzilate, Systox, and lindane were moderately toxic. Toxaphene and ovex were not as toxic as the preceding formulations. Heptachlor and dieldrin were only slightly toxic at 0.1%. Ryania, benzal azine and Aramite were almost innocuous. Systox was also toxic systemically; schradan was not. Lord (1949), Clancy (1952–54) and MacPhee & Sanford (1954) have recently substantiated, in field trials, the high toxicity of DDT, the phosphorous compounds and Chlorobenzilate to typhlodromid mites. Clancy (1954), also, found that Aramite was less toxic than ovex and that ryania was nontoxic.

Puratized agricultural spray, a phenyl mercuric triethanol ammonium lactate, was the most toxic fungicide. From other work done with high solvent ratio formulations we might infer that the solvent may have contributed to the toxicity. In preliminary unpublished data another mercury fungicide, phenyl mercuric acetate, was nontoxic to *T. fallacis*. Bordeaux mixture, glyodin, captan and maneb were moderately toxic at 0.2%, a high concentration. Zineb, ferbam, sulfur and tribasic copper sulfate were only slightly harmful at the high dosage to the adult mites.

Some of these comparisons can be misleading if improper interpretations are made from preliminary data. Cutright (1944) had demonstrated the high toxicity of sulfur and ferbam to some species of predaceous mites; Mac-

Table 1.—Contact toxicity[a] of some pesticides to *Typhlodromus fallacis*. Ohio State University.

MATERIAL	ACTUAL PERCENTAGE CONCENTRATION	PERCENTAGE MORTALITY (24-HR.)
April, 1953, tests		
Malathion—25 EM	0.005	100
	.00125	100
Parathion—15 WP	.005	100
	.00125	100
DMC—25 EM	.005	97
	.00125	75
DDT—25 WP	.01	100
	.0025	90
Chlorobenzilate—25 WP	.01	92
	.0025	45
Systox—25 EM	.01	82
	.01[b]	100
Lindane—25 WP	.04	95
	.02	91
Ovex—50 WP	.05	80
	.01	35
Toxaphene—60 EM	.04	76
	.02	44
Heptachlor—25 EM	.5	100
	.1	40
Dieldrin—18.5 EM	.5	88
	.1	56
Schradan—45	.01	50
	.01[b]	0
Dutch Sulfur—25 WP	.5	12
Aramite—15 WP	.4	5
Benzal azine (Collodial dispersion)	.05	5
Water Check	—	12
March, 1954, tests		
Puratized Agr. Spray—5	0.2[c]	96
	.02	88
	.002	35
Captan—75 WP	.2	55
	.02	22
Glyodin	.2	61
	.02	22
Maneb—50 WP	.2	49
	.02	41
Bordeaux—12.75 DB	.2[d]	70
	.02	12
Fermate 76	.2[d]	19
Tribasic copper sulfate—50 WP	.2	20
Zineb—65 WP	.2	20
Sulfur—95 WP	.2	2
Ryania—100	.2[d]	0
Water Nacconol Check	—	12

[a] Leaf dip method; two replicates; 50–100 mites in 1953, 50 to 120 mites in 1954.
[b] Systemic.
[c] Phenyl mercuric triethanol ammonium lactate.
[d] Nacconol added as a wetting agent at 200 p.p.m.

Table 2.—Comparative contact toxicity[a] of eight pesticides to *Typhlodromus fallacis*. Ohio State University. April, 1953.

Material	P.P.M. To Give 24-Hour LD_{50}	P.P.M. To Give 24-Hour LD_{90}
DMC—25 EM	5	22
DDT—50 WP	50	250
TDE—50 WP	85	370
Methoxychlor—50 WP	250	1,000
Chlordane—75 EM	600	3,000
Heptachlor—25 EM	1,500	7,800
Aldrin[b]—25 EM	1,900	9,000
Dieldrin—18.5 EM	2,200	10,000

[a] Average 6 replicates in three series.
[b] Laboratory preparation.

Phee & Sanford (1954) had also demonstrated the harmful effects of these two fungicides to species of typhlodromid mites in Nova Scotia. In this case there is an apparent conflict between field and laboratory results. These anomalies can be resolved if we examine the effects of chemicals on other stages of the life history (table 5).

Table 2 shows comparative 24-hour contact toxicity data for DDT, some of its analogs and some terpene analogs. DMC was the most toxic; it was five times as toxic as DDT to the motile stages. Dieldrin was the least toxic in the series. Data in table 3, however, show that although dieldrin was seeminlgy only slightly toxic as an initial contact poison, even at 0.05%, it is a slow acting toxicant. Consequently, if 7-day counts are made, dieldrin is toxic at high dosages. Clancy (1954) in his field experiments rated dieldrin as a moderately harmful pesticide against typhlodromid mites.

Lead arsenate was innocuous to the adults and the larvae (table 3) but it interfered with the egg production at the higher dosages. Benzal azine, as an emulsion, from a concentrate, was moderately toxic. Here again, the experiment measures the toxicity of the adjuvants. Although the contact mortality was high, the residual toxicity was

Table 3.—Contact[a] and residual toxicity of several pesticides to *Typhlodromus fallacis*, June, 1954.

Material and Per Cent Concentration	No. of Mites Live	No. of Mites Dead	Per Cent Control (24-Hr.)	No. (After 4 Days) of Live Mites	No. (After 4 Days) of Eggs	No. of Live Mites (10 Days)
Lead arsenate—96 WP						
0.2	73	2	3	103	53	Many
.05	72	2	3	72	128	Many
Benzal azine—20 EM						
0.2	6	142	96	2	93	Many
.05	5	48	89	0	7	Many
.01	38	22	36	57	41	Many
Ovex—50 WP						
0.2	5	108	95	0	0	None
.05	12	51	80	0	0	None
.01	11	13	54	8	0	None
Dieldrin—15 EM						
0.2	17	81	81	3	3	None
.05	68	45	41	22	3	Many
.01	55	15	25	70	44	Many
Aramite—15 WP						
0.2	0	70	100	0	0	None
.05	33	46	58	2	0	Few
.01	46	41	59	0	0	Few
Water check	74	5	6	89	128	Many

[a] Plastic-cup method.

Table 4.—Contact[a] and residual toxicity of several pesticides to *Typhlodromus fallacis*, August, 1954.

Material and Per Cent Concentration	Per Cent Control (24-Hr.)	No. of Eggs	Per Cent Control—No. of Eggs (5 Days)		No. of Forms (10 Days) Adult	Immature	Eggs
Lead arsenate 96 WP							
0.1	4	45	0	30	52	40	36
.05	0	44	0	67	—	—	—
Fermate—76							
0.2	20	42	0	29	39	8	29
.1	10	58	0	78	32	17	23
Glyodin (Tech.)							
0.1	98	13	0	—	—	—	—
.01	56	29	0	—	—	—	—
Sulfur—95 WP							
0.25	10	34	0	10	10	2	10
.125	0	41	0	13	11	4	12
.062	0	41	0	34	27	11	30
DDT—50 WP							
0.01	89	10	100	0	0	0	0
Solvent check	15	26	0	19	18	11	25

[a] Plastic-cup method.

low for the azine. Ovex and Aramite, although only moderately toxic to the adults, were highly toxic to the larvae at the higher dosages and ovex was a powerful ovicide. Dieldrin was approximately as toxic as Aramite, as a contact poison, and it showed residual toxicity at very high levels. This table presents data which are more reliable for predicting field performance of the chemicals.

Table 4, again shows that although lead arsenate was not toxic to the motile stages, it depressed egg production at high dosages. Clancy (1954), however, found that lead arsenate was moderately toxic in some situations to *Typhlodromus* spp. Ferbam and sulfur were not toxic to the adults but sulfur was toxic to the immature stages. Glyodin was very toxic at 1000 p.p.m. but only moderately at 100 p.p.m. and showed no residual action. DDT was highly toxic as a contact and as a residual poison.

There were indications from the previous "leaf spray plastic cup technique" that sulfur was toxic to the immature stages. Therefore sulfur was evaluated more critically by the "petri dish detached leaf sticky barrier" method. All motile forms were killed by a 50 p.p.m. TEPP pretreatment.

The figures in table 5 show that sulfur was toxic to the larvae and it was ovicidal. Ferbam was slightly poisonous to the larvae but not to the eggs. Ferbam does not adhere to the eggs or disperse too well unless a wetting agent is used. Several stickers and wetting agents were tried in the ferbam preparations to increase adhesion since there was some evidence that ferbam was ovicidal if a visible deposit was present on the egg. A satisfactory answer, however, was not obtained. Lead arsenate was innocuous, again. Clancy, however, as previously stated, found lead arsenate toxic to some *Typhlodromus* spp. and Lord (1940) proved that lead arsenate was harmful to the predaceous mite, *Anystis agilis* Banks, but not to *Mediolata novaescotiae* Nesbitt.

Data given in table 6 show that captan, glyodin and ferbam at lower dosages were only slightly toxic to the immature stages. DDT and ovex were highly toxic at 500 p.p.m. whereas dieldrin was only slightly poisonous. In this test Aramite was the least toxic.

Table 5.—Toxicity of sulfur, ferbam and lead arsenate to the eggs and larvae of *Typhlodromus fallacis*. 1955.[a]

Material and Per Cent Concentration	Pretest Egg Count	No. of Eggs (48-Hr.)	Per Cent	
			Eggs Unhatched	Mortality (48-Hr.)
	January 22			
Sulfur—95 WP				
0.25	192	52	26	85
.125	235	58	37	83
.063	73	38	46	76
Ferbam—76[b]				
0.25	88	24	14	22
Lead arsenate—96 WP				
0.25	101	16	8	13
TEPP check	87	12	13	20
	February 9			
Sulfur—95 WP				
0.125	96	18	19	81
Lead arsenate—96 WP				
0.125	63	0	0	20
TEPP check	168	10	6	0

[a] Tested by the Jeffers-Ristich spray-booth method; 90 ml. for 45 sec. at 30 r.p.m. and 40 p.s.i.
[b] Ultrawet added; ball-milled 30 minutes.

In the next test (table 7), one set of leaves was dipped in a 32-p.p.m. DDT emulsion and another set in a 50-p.p.m. TEPP solution. After the water had evaporated, the leaves were sprayed with the other toxicants. Three replicates were used and the eggs were 24 hours old. Ferbam and dieldrin were more toxic to the immature

Table 6.—Toxicity[a] of eight formulations to the eggs and larvae of *Typhlodromus fallacis*. March 10, 1955.

Material and Per Cent Concentration	Pretest Egg Count	No. of Eggs (72-Hr.)	Per Cent	
			Eggs Unhatched	Mortality
Ryanicide[b]—100				
0.1	74	0	0	0
Ferbam[b]—76				
0.125	63	1	2	38
DDT[c]—Tech.				
0.05	56	7	13	97
.01	63	1	2	44
Dieldrin[a]—Tech.				
0.05	61	0	0	21
.01	71	0	0	5
Aramite—15 WP				
0.01	80	1	1	7
Ovex—50 WP				
0.01	52	2	4	85
Captan—75 WP				
0.05	88	4	4	27
Glyodin[d]—Tech.				
0.05	49	3	6	18
TEPP check	73	0	0	0

[a] By the Jeffers-Ristich spray booth method.
[b] Ultrawet added; ball-milled 30 minutes.
[c] Prepared with acetone and Triton X-155.
[d] Prepared with isopropanol and ultrawet.

Table 7.—Influence of a pretreatment with DDT on the toxicity of other pesticides to *Typhlodromus fallacis*. 1955.

Material and Per Cent Concentration	Pretest Egg Count	No. of Eggs (72-Hr.)	Per Cent		No. of Mites (10 Days)
			Eggs Unhatched	Apparent Mortality	
Dipped in an emulsion containing 32 p.p.m. of DDT[a] (March 17)					
DDT Check	96	1	1	16	Few
Ferbam—76					
0.25	76	12	15	64	Few
Dieldrin—Tech.					
0.05	75	2	4	83	None
.01	82	4	4	33	Few
Leaves dipped in TEPP (50 p.p.m.)[a] (March 17)					
TEPP Check	49	1	2	0	Many
Ferbam					
0.25	87	12	14	36	None
Dieldrin-Tech.					
0.05	66	2	3	27	None
.01	80	1	2	7	Many
DDT—Tech.[b]					
0.05	70	2	3	100	None
.01	70	7	10	16	Few
Aramite[b]					
0.01	92	2	3	19	Many
Ovex[b]					
0.01	70	2	4	50	Few
All leaves treated with 50 p.p.m. TEPP; then one set sprayed with 100 p.p.m. DDT (March 31)					
DDT Check	64	0	0	22	
Dieldrin—Tech.					
0.05	95	0	0	45	
Aramite—15 WP					
0.01	106	0	0	14	
Ovex					
0.01	139	1	0	51	
Ferbam					
0.25	115	6	5	44	
TEPP Check	115	0	0	5	
Dieldrin—Tech.					
0.05	120	0	0	24	
Aramite—15 WP					
0.01	134	3	2	7	
Ovex—50 WP					
0.01	178	1	0	44	
Ferbam—76					
0.25	117	4	3	60	
Rohn & Haas FW 293-25 WP					
0.05	64	2	3	100	

[a] Hour later sprayed with pesticide (70 ml./40 p.s.i./30 r.p.m.).
[b] Not included in comparison.

stages in the pretreated DDT series. Aramite and ovex were included only for additional comparative data. Ferbam was slightly ovicidal.

In a subsequent test (table 7) where the leaves were pretreated with a 100-p.p.m. DDT emulsion, the toxicity of ferbam or dieldrin was not enhanced. Ferbam, however, was moderately toxic in both series. The Rohm & Haas FW 293 was highly toxic at 500 p.p.m. The data in table 8 shows that FW 293 was more toxic than DDT and that DDT was more toxic than dieldrin to the immature stages.

SUMMARY.—These experiments represent the first attempt to evaluate the effect of many pesticides, through several new testing techniques, on the predaceous mite, *Typhlodromus fallacis* (Gar.).

The most toxic insecticides were the phosphorus-type

Table 8.—A comparison[a] of dieldrin, DDT, and Rohm & Haas FW 293 against the immature stages of *Typhlodromus fallacis*.

MATERIAL AND PER CENT CONCENTRATION	PRETEST EGG COUNT	APPARENT PER CENT MORTALITY—(72 HR.)
Dieldrin—Tech.		
0.1	97	48
.08	148	24
.06	155	12
DDT—Tech.		
0.04	108	40
.02	88	33
.01	72	8
Rohm & Haas FW 293—25 WP		
0.02	85	90
.01	80	26
TEPP check	164	2

[a] Pretreated with TEPP and sprayed.

compounds and the DDT analogs. Ryania, dieldrin and lead arsenate were the least toxic although lead arsenate seemingly interfered with egg production. DMC was the most toxic acaricide and Chlorobenzilate, Rohm & Haas FW 293 and ovex were moderately poisonous. The latter two were also ovicidal. Aramite was the least toxic acaricide but like ovex it was ovicidal and had residual toxicity at higher levels.

Sulfur was the most toxic fungicide to the immature stages and it was ovicidal. Phenyl mercuric triethanol ammonium lactate was the most toxic fungicide to the adult mites. The carbamates, glyodin and captan were slightly to moderately toxic. Ferbam gave erratic results. Certain evidence, however, points up its ovicidal action and harmful effects to the larvae.

REFERENCES CITED

Clancy, D. W., and H. N. Pollard. 1952. Effect of DDT on mite and predator populations in apple orchards. Jour. Econ. Ent. **45**: 108–14.

Clancy, D. W. 1953. Preliminary results in codling moth control with ryania insecticides. [Mimeographed]

Clancy, D. W. 1954. Correspondence.

Cutright, C. R. 1944. Population of European red mite as effected by spray schedules. Jour. Econ. Ent. **37**: 499–502.

Daugherty, D. 1953. A partial survey of the insecticidal tolerances of the predaceous mite, *Typhlodromus fallacis* (Garman). Master's Thesis, Ohio State University.

Herbert, H. J. 1952. Progress report on predacious mite investigations in Nova Scotia. Ann. Rept. Ent. Soc. Ontario **83**: 27–29.

Huffaker, C. B., and C. E., Kennett. 1953. Differential tolerance to parathion of two *Typhlodromus* predatory on the cyclamen mite. Jour. Econ. Ent. **46**: 707–8.

Lord, F. T. 1949. The influence of spray programs on the fauna of apple orchards in Nova Scotia. III. Mites and their predators. Canadian Ent. **81**(8): 202-14: (9): 217–30.

MacPhee. A. W., and K. H. Sanford, 1954. The influence of spray programs on the fauna of apple orchards in Nova Scotia. VII. Effects on some beneficial arthropods. Canadian Ent. **86**: 128–35.

Pickett, A. D., and N. A. Patterson. 1953. The influence of spray program on the fauna of apple orchards in Nova Scotia. A review. IV. Canadian Ent. **85**: 472–8.

Ristich, S. S. 1956. Mass rearing and testing techniques for *Typhlodromus fallacis*. (Gar.) Jour. Econ. Ent. **49**(4): 476–9.

Stevenson, J. O. 1955. The toxicity of several chemicals to the predaceous mite, **Typhlodromus fallacis** (Garman), and notes on its biology. Master's Thesis, Ohio State University.

Biology of the Winter Grain Mite and Its Control in Small Grains[1]

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The winter grain mite (*Penthaleus major* (Dugés)) damaged fall-sown small grains in north-central and central Texas during the winters of 1952 and 1953. Similar damage has been observed for many years, and some people attributed it to freezing weather. The county agent in Dallas County reported damage by this mite as early as 1919. Information on control has been requested by farmers and county agents. Since there was no available information on the biology and control of this mite in small grains, in 1952 a research program was undertaken at the Denton substation of the Texas Agricultural Experiment Station.

PREVIOUS WORK.—The winter grain mite was first described, probably in France, by Dugés in 1834 as *Tetranychus major*. The first record in the United States was by Banks (1902), who described it as a new species, *Notophallus dorsalis*. The first reference to it as a pest of economic importance was by Marchal (1908) in France, were it was damaging peas. In the United States it was first reported of economic importance in 1911, when R. N. Wilson found it damaging barley in Arizona (unpublished manuscript). Many of Wilson's observations agree closely with those made by the author.

Banks (1917) reported that the mite specimens he had received from Arizona on December 14, 1911, were a new species, *Notophallus viridis*. Froggatt (1921) observed the

[1] Accepted for publication January 18, 1956. Much of the information on biology in this paper was taken from an unpublished manuscript entitled "The barley mite attacking cereal and forage crops, *Notophallus viridis* Banks," by T. Scott Wilson (deceased) of the former Bureau of Entomology and Plant Quarantine. Mr. Wilson's research was carried on mainly in Arizona from 1911 to 1915.

[2] In cooperation with the Texas Agricultural Experiment Station. The assistance of M. J. Norris, associate agronomist, at the McGregor substation, in obtaining field history data and in making acaricidal tests at that station is gratefully acknowledged.

mite damaging oats in Australia, and described it as a new species, *N. bicolor*. He gave it the common name "blue oat mite" and wrote a description of the species. Andre (1932) described the mite and stated that it was very destructive to peas and other vegetables in France. Womersley (1933) reported finding it in South Africa and Australia; he also described the mite. Swan (1934) reported the mite as being troublesome on large canary grass (*Phalaris tuberosa*) and subterranean clover, and discussed characters by which it might be distinguished from closely related species. The synonymy of the mite was discussed by Womersley (1935), and he concluded that its proper name is *Penthaleus major* (Dugés).

Essig (1939) reported collecting specimens of the mite near San Jose, Calif., in 1912, where it was attacking a springtail, *Achorutes armatus* Nicolet, that was swarming on the surface of fresh water; he later found it on peas. Campbell (1941) observed it in California between 1912 and 1916, and concluded that it might become a serious pest of peas. Womersley (1941) published a key to the four genera of *Penthaleidae* Oudemans, one of which is *Penthaleus*. Baker (1946) discussed the synonymy of specimens collected in North and Central America, and also published a key to the four genera of the family *Penthaleidae*. Baker & Wharton (1952) placed *Penthaleus* back into the family *Eupodidae* Koch, and discussed characters useful for separating this genus from other closely related genera.

CLASSIFICATION.—The mite belongs to the family *Eupodidae* Koch, 1842, formerly *Penthaleidae* Oudemans, 1831, and it consists of seven genera. *Penthaleus* is easily separated from the other genera, in that the anus is dorsal, whereas in *Halotydeus* it is terminal and in all other genera it is ventral (Baker & Wharton 1952). It has been referred to as the "blue oat mite" and the "pea mite" in the literature, but since it occurs during the winter, is not blue, and causes more damage to the small grains than other crops, the common name "winter grain mite" seems more appropriate. Farmers commonly call it the "red spider mite" because of its red legs and mouth parts, but its habits and the damage it does are considerably different from those of the common red spiders, the Tetranychidae.

Various scientific names have been given to the winter grain mite. Based on the synonymy of Womersley (1935) and Baker (1946), the following synonymy, as approved by E. W. Baker on May 13, 1954, has been developed:

Penthaleus major (Duges) 1834
Tetranychus major Duges 1834
Penthaleus haematopus Koch 1835
Penthaleus erythropus Koch 1835
Penthaleus insulanus Thorell 1872
Notophallus dorsalis Banks 1902
Notophallus viridis Banks 1917
Notophallus bicolor Froggatt 1921
Penthaleus bicolor Womersley 1933

GEOGRAPHICAL DISTRIBUTION.—The winter grain mite is widely distributed, particularly throughout the Temperate Zones. It has been recorded from the United States, Germany, Italy, and France, in the North Temperate Zone; and from Australia, New Zealand, and South Africa in the South Temperate Zone.

In the United States there are records from Texas, Oklahoma, Kansas, Missouri, Arizona, California, Tennessee, District of Columbia, Pennsylvania, Massachusetts, New York, and Minnesota. In Texas the heaviest infestation is confined to the north-central and central small-grain areas and to the Comfort-Kerrville area in the Southern part of the State.

In Australia the records are from New South Wales, South Australia, Victoria, Federal Territory, and Western Australia.

HOST PLANTS.—Small grains and grasses are the favored hosts of the winter grain mite, but it also infests and damages legumes, vegetables, and weeds. Hosts observed or recorded in the literature are as follows: oats, wild oats, barley, little barley, wheat, rye, sweetclover, clover, burclover, lupine, alfalfa, peas, lettuce, wild lettuce, prickly lettuce, potato, bluegrass, Johnson grass, rescue grass, large canary grass, wild mustard, chess, ragweed, common mallow, pigweed, shepherds-purse, dropseed, perennial ryegrass, and thistle.

NATURE OF INJURY.—Heavily infested fields have a grayish or silvery appearance. This condition is caused by the removal of chlorophyll and plant juices from the leaves by the feeding mites. When large numbers feed on the plants, the tips of the leaves turn brown and die; and when feeding continues for several days, the entire plant dies. These mites do not cause the yellowing characteristic of the feeding by tetranychid mites. Many of the infested plants do not die, but they become stunted and produce little forage or grain. Damage is more severe on young plants than it is on large, healthy plants.

There are two types of damage to the small grains—reduced amount of forage throughout the winter and reduced yields of grain in the spring and summer. Where winter grazing is practiced, the reduction in forage probably is of the greater importance, because farmers rely on the small grains for fall and winter pasture for livestock.

DESCRIPTION.—The egg (Fig. 1) is kidney-shaped, and when freshly laid it is plump and reddish-orange. Within a few minutes the tough outside membrane becomes wrinkled and turns pale, and in a few days it becomes straw-colored. Based on the measurements of 65 eggs, the length averaged 0.25 mm. and the diameter 0.14 mm.

The larva (Fig. 2) is reddish-orange when first hatched. It averages 0.18 mm. in length and 0.11 mm. in width. The body is sparsely covered with whitish setae. There are three pairs of legs. After feeding for about 1 day, the body becomes light brown, and an irregular lighter spot surrounds the dorsally placed anus. The legs and mouth parts are yellowish-orange. Just before molting the body becomes a darker brown, tinged with green.

The nymph is similar to the full-grown larva except for having four pairs of legs. The fourth pair appears back of the third pair after the first molt. The nymphal stage has three instars, which are similar in appearance except for the increase in size. The first and fourth pairs of legs are longer than the second and third. The body is dark brown with a light-brown spot surrounding the dorsally placed anus. The legs and mouth parts are yellowish-orange. Just before each molt the body becomes plump and tinged with green, and the legs become light pink.

The adult (Fig. 3) is dark-brown to black, tinged with green. In certain lights it may appear to be purplish-blue. It averages 1 mm. in length. The body is composed of a gnathosoma (region of the oral opening) and an idiosoma

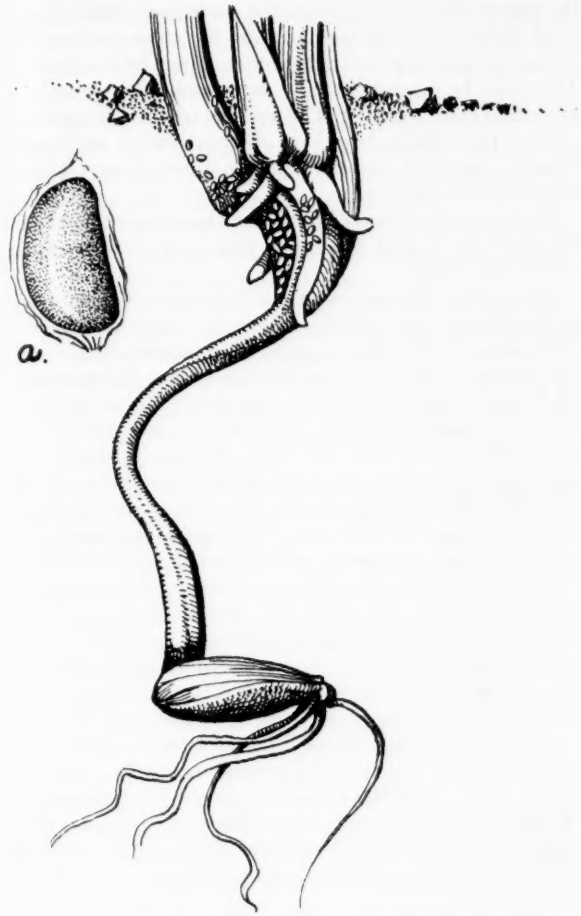

FIG. 1.—Winter grain mite eggs on crown and sheath leaves of barley. (a) Enlarged egg.

(region of the legs and the posterior part). Three transverse lines are noticeable on the dorsal part of the body, the anterior one being more distinct than the others. A small silvery eye is present on each side of the idiosoma, a little to the rear of the second pair of legs. The anal aperture is located on the dorsum about one-third the length of the body from the apex of the abdomen, and it is usually surrounded by a reddish-orange spot. A droplet of clear to light-yellow fluid is frequently found exuding from the anus of living specimens. The body is sparsely covered with very small, white setae. The chelicerae are short, and each has two downward-curved teeth on the distal end, which forms a beaklike apparatus for piercing plant tissues. The pedipalps are four-jointed and short. The mouth parts and legs are reddish-orange. The first and fourth pairs of legs are longer than the second and third pairs. The legs are divided into six segments. The tarsi are small with two claws and a haired empodium. There is a spine on the inside of the tibia near the tarsal joint. The legs are covered with white hairs. The genital

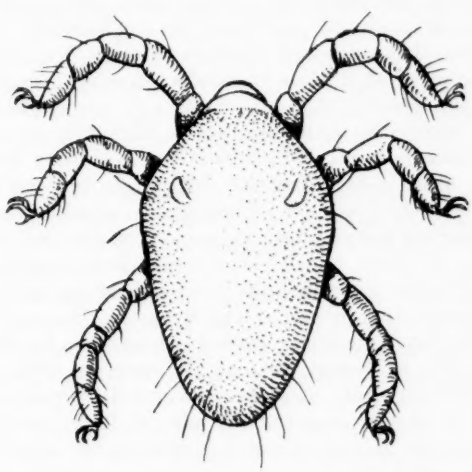

FIG. 2.—Winter grain mite larva.

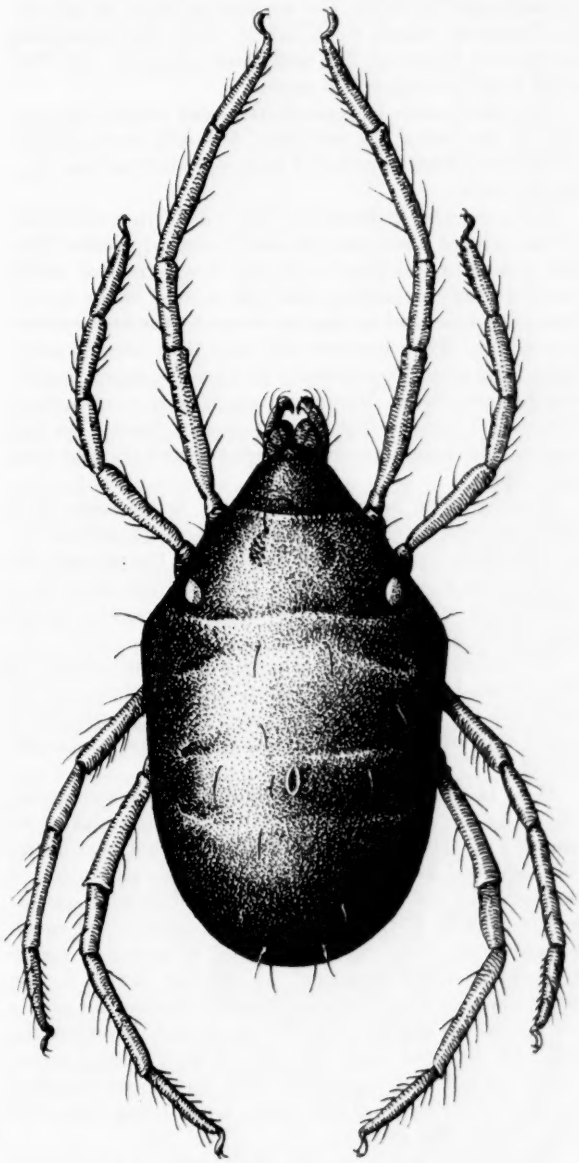

FIG. 3.—Winter grain mite adult female.

opening is ventral, and it is bordered by two pairs of eliptical suckers.

Males have been observed and described by several authors. T. Scott Wilson (unpublished manuscript) described the males as being somewhat smaller than the females, with the genital opening shorter and less curved. Andre (1932) quoted Oudemans as stating that the length of the males is less than 700 microns, whereas that of the females is more than 788 microns. Womersley (1933) reported that males of this species had not been discovered. Males have not been observed by the author, and their occurrence has not been definitely established.

LIFE HISTORY.—The length of the several stages in the life history of this mite varies with the weather. Under observed optimum conditions the average time from oviposition to death of the adult was 98.1 days, not including the period of aestivation of the eggs. The incubation period averaged 25 days. The average numbers of days in the immature stages were: larval, 12.2; first nymphal, 8.6; second nymphal, 7.8; and third nymphal, 7.0. The adult stage averaged 37.5 days.

The exact mode of reproduction and sexual relationship of the mites has not been definitely worked out. Womersley (1933) concluded that reproduction was parthenogenetic.

SEASONAL DEVELOPMENT.—There are two generations of the winter grain mite in north-central Texas. The first generation develops in the fall from eggs that aestivated during the summer, and the second in the spring from eggs deposited during the winter by the first-generation adults. Mites are generally present in infested grain fields from about November 1 to April 15, depending on weather conditions. Maximum populations occur around February 1 for the first generation and April 1 for the second. The second-generation mites deposit the eggs that aestivate.

HABITS.—The larvae become very active soon after hatching, and after several hours they begin to feed on the sheath leaves or tender shoots near the ground. At night or on cloudy days when there is little wind they feed higher on the plants. The first molt marks the change from larva to nymph.

The habits of the small nymphs are similar to those of the larvae, but in the last nymphal stage they become more like the adults and feed higher on the plants. They avoid the bright light and heat of the sun, as do the adults.

Most of the feeding by the adults is done during the night. As the sun rises the mites descend the plants, remaining on the shaded sides. During the hot part of the day, between about 10 A.M. and 4 P.M., they can be found on the moist soil surface under foliage if there is considerable growth. If the soil is dry and there is not much foliage cover, they dig into the soil in search of cooler temperatures and moisture. As the sun lowers in the afternoon and shadows form, the mites ascend the plants to feed on the shaded sides. At sunset and thereafter the plants in infested fields are covered with feeding mites. With the aid of a searchlight they have been observed feeding at all hours of the night. Upon being disturbed they drop to the ground.

The female mites deposit their eggs on the sheath leaves and stems and on and in the soil near the base of the plants (Fig. 1). Those on the leaves are usually fastened to the inner surface next to the stem and on the stems by a mucilaginous substance secreted by the female. They may be deposited singly, but usually large numbers are found close together. The average number of eggs deposited per female, based on observations of six reared females, was 31.3, and the largest number observed for a single female was 65.

Excreta in the form of bubbles can be seen exuding from the anal opening of the mites and on the leaves upon which they feed.

EFFECTS OF ENVIRONMENTAL FACTORS.—Temperature and moisture are probably the most important factors influencing mite development and abundance. Cold, rather than warm, temperatures favor their development. Mites are present in the field during the entire winter in Texas. Oviposition is heaviest between 50° and 60° F., temperatures between 45° and 55° are optimum for hatching, and adult activities are greatest between 40° and 75°. When temperatures drop below or go above these extremes, the mites stop feeding and descend to the ground or burrow into the soil. When the daily temperatures exceed 75° in the spring, mite activity drops rapidly and the eggs do not hatch. The aestivating eggs do not hatch in the fall until there is adequate moisture in the form of rain. On hot, dry days it was necessary to dig into the soil to a depth of 4 or 5 inches to find mites, where they had gone seeking moisture and relief from the heat.

The mites are not harmed by sleet or ice cover or by ground frozen to a depth of several inches for short periods. Such conditions were observed in north-central Texas, and with warming and thawing the mites were again present in large numbers. They burrow into the soil to avoid the cold just as they do to avoid the hot temperatures.

Light, loose soils are favorable for mite development. The mites cannot survive in soils that are packed by heavy grazing or in those that have a hard surface crust. During the winter of 1953–54 a potentially damaging population was practically eliminated in many fields in north Texas following the formation of such a crust after rains.

DISPERSION.—Spread from field to field is probably due to the transportation of aestivating eggs on debris, such as grain stubble and leaves, on soil adhering to implements that are moved about, or on forage or straw carried from infested fields in livestock feeding operations. It is also possible that aestivating eggs are wind-blown from infested to noninfested fields. Migration in a field by immature forms and adults is due to scarcity of food and is usually only a few rods. Migrations to grain fields have been observed from adjoining areas where there were undisturbed aestivating eggs, such as fence rows, straw stacks, or other uncultivated areas. Rescue grass appeared to be the favored host in such situations in Texas.

BIOLOGICAL CONTROL.—The larvae of *Chrysopa californica* Coq. have been recorded as feeding on the winter grain mite at Tempe, Arizona (Wildermuth 1916). The red predatory mite *Balaustium* sp., family Erythraeidae, feeds on several stages of the mite in Texas, but there has been no indication that it is of economic importance in its control.

CULTURAL CONTROL.—Cropping practices have a

marked effect upon the occurrence and damage caused by the winter grain mite. During the winter of 1952–53 it was observed that not all fields were infested and that damage in infested fields varied. An examination of the debris in the fields showed that cropping practices were responsible for the variation. Field history, infestation, and damage data from 61 grain fields examined at random showed that the following conditions existed:

(1) In fields that were in small grains continuously for three or more years the infestation was heavy, usually with extensive damage.
(2) In fields that were in small grains for two consecutive years, the infestation was light and of the spotted type, and damage was light.
(3) In fields where the previous crop was other than a small grain, such as cotton, corn, clover, or sorghum, mites were either absent or very scarce and there was no damage.

Practices that would eliminate the continuous cropping of small grains should therefore reduce infestations and damage by this mite.

CHEMICAL CONTROL.—Marchal (1908) recommended heavy applications of potassium or calcium sulfate for the control of the mite on peas. In more recent tests on field peas in California, Middlekauff & Pritchard (1949) found that dusts of DDT, parathion, and TEPP, and a parathion spray at rates of 0.6, 0.23, 0.21, and 1.2 pounds of toxicant per acre gave satisfactory control.

Preliminary tests with acaricides for the control of this mite were made by the author in Texas in 1952. The sprays were applied with a compressed-air sprayer at the rate of 10 gallons per acre. The plots were 20 by 50 feet and were not replicated. Parathion and malathion were applied at 0.25 pound and Aramite at 0.5 pound per acre. Three days later the control was 100% for parathion, 92 for malathion, and 81 for Aramite. Further tests in 1952 were precluded by the lateness of the season and the high temperatures that followed.

Three tests with acaricides were made during 1953. They were applied in emulsions at the rate of 10 gallons per acre. A portable sprayer powered by a 1-hp. motor was used at a pressure of 50 pounds per square inch. It was equipped with a gear pump, pressure regulator, and a spray boom with four nozzles. In all tests there were three replications and a check. Each plot was 12 by 36 feet, and the borders were 50 feet wide. Mite counts were made just before spraying and 3 days afterward. The sampling unit was 1 foot of row. Mites were counted on the plants, under the foliage on the soil surface, and in the top soil layer.

The first test was made in a field of oats that averaged 3 inches in height on the Bingham farm at Denton, on January 28. The day was clear with little wind, and the temperature was 63° F.

The second test was made on February 3 on Bluebonnet farm, substation No. 23 of the Texas Agricultural Experiment Station at McGregor. The oats averaged about 5 inches in height. The day was clear with a temperature of 75° F. and a wind velocity of 15 to 25 miles per hour.

A late infestation in wheat about 12 inches tall on the James Griffin farm at Plano offered an opportunity to test the effectiveness of several acaricides in tall grain and at reduced dosages. This test was made on March 23 on a clear day with the temperature 75° F. and the wind velocity 5 to 25 miles per hour.

The results of these tests are presented in table 1. All the organic phosphorus compounds tested—parathion, TEPP, Metacide, malathion, and Systox—gave better than 97% control. Good results were also obtained with the organic sulfur compounds—ovex, Sulphenone, and Aramite.

Table 1.—Control of the winter grain mite in Texas, 1953.

Acaricide	Pounds of Toxicant per Acre	Location of Field	Number of Mites per Foot of Row		Per Cent Control
			Before Treatment	After Treatment	
Parathion	0.25	McGregor	46	0	100
		Plano	105	0	100
		Denton	147	0.8	99
	.125	McGregor	84	0	100
Systox	.5	McGregor	54	0	100
Metacide	.25	McGregor	90	0	100
		Denton	341	0.2	100
TEPP	.25	McGregor	35	0.3	99
		Denton	164	3	98
	.125	McGregor	42	0	100
Malathion	.25	McGregor	19	0.3	98
Aramite	.5	McGregor	43	0.8	98
	.33	Plano	112	10	89
Ovex	.5	McGregor	90	5	95
		Denton	182	32	78
Sulphenone	.5	McGregor	38	3	92
	.33	Plano	98	6	92
Chlorobenzilate	.25	McGregor	41	25	42
Checks		Denton	171	137	—
		McGregor	81	84	—
		Plano	127	103	—

The complete absence of mites in some cases following spraying with phosphorus compounds led to the belief that the daytime counts might not be reliable, since the mites feed mainly at night. Therefore, 2 days after the spraying at McGregor counts were made in the plots at 8 P.M. with the aid of a searchlight. At this hour the mites were feeding in full force in unsprayed areas. A comparison of these counts with those made in daylight at 4 P.M. on the following day showed little difference in the populations. It was concluded, therefore, that the absence of mites in the plots sprayed with phosphorus compounds was due to their toxic action.

A single application of parathion or TEPP applied by farmers at a suggested dosage of $\frac{1}{4}$ pound per acre provided adequate mite control in small grains. Counts were made in eight fields sprayed by farmers before treatment and 3, 6, 15, and 30 days after treatment. There was no buildup in infestation in any of the fields during the period they were observed. The control was satisfactory even when spraying was done at temperatures below 50° F., when there were strong winds, or where coverage was poor because of faulty spraying equipment.

CONCLUSIONS.—A change in cultural practices from continuous cropping of small grains to a rotation with another crop at least every 2 years will eliminate or greatly reduce damage by the winter grain mite. Crop rotation has been and is being recommended as an improved farm practice by State agricultural experiment stations and other agricultural agencies. The effective control of the winter grain mite by crop rotation adds emphasis to the value of rotations.

Although the tests with acaricides have been limited,

it is believed, on the basis of results thus far and information on practical field usage, that the phosphorus compounds parathion, TEPP, or malathion at ¼ pound per acre could be considered for recommendation for control of this mite in small grains. Systox is a systemic insecticide and could not be recommended until further knowledge of its action in the plant and residual effects has been obtained. The organic sulfur compounds ovex, Sulphenone, and Aramite showed promise of giving good control, but more data on their effectiveness are needed.

Caution.—Parathion and TEPP are highly toxic to man and livestock. They should be handled with great care, and all directions and precautions printed on the containers should be strictly observed. Both should be applied with power machines only and by persons experienced in handling and applying poisonous chemicals. Animals should not be pastured in grain fields for 2 weeks after they have been treated with parathion or malathion. TEPP residues disappear more quickly, and the fields treated with this acaricide can be used as pasture after 3 days.

SUMMARY.—The winter grain mite (*Penthaleus major* (Dugés)) has damaged fall-sown small grains in north-central and central Texas. Infested fields have a grayish or silvered appearance, caused by the removal of juices and chlorophyll from the leaves, with consequent reduction in forage during the fall and winter and reduced yields of grain.

The mites hatch in the fall from aestivating eggs and are present in infested fields in Texas throughout the winter. Cold temperatures and moisture are necessary for their development. The mites die with the approach of hot weather in the spring. Two generations a year have been observed. Most of the feeding is done at night or on cloudy days.

Small grains or grasses are favored hosts, but the mites have been observed on vegetables and weeds. Little preference was shown for wheat, oats, or barley.

Dispersion is accomplished by the transportation of aestivating eggs on grain stubble and leaves, other debris, or soil involved in the movement of farm machinery from field to field. Strong winds probably blow the aestivating eggs about.

Fields continuously in small grains are most heavily infested. Crop rotation has greatly reduced or eliminated damage by this mite, and is recommended to the grower.

The phosphorus compounds parathion, TEPP, Systox, Metacide, and malathion gave satisfactory control of the mite at relatively low dosages. The organic sulfur compounds ovex, Sulphenone, and Aramite also showed promise of controlling the mite. In fields sprayed by farmers, one application of either parathion or TEPP at ¼ pound per acre gave seasonal control of the mite. Either of these acaricides or malathion at the same dosage is recommended for the control of the winter grain mite in small grains.

REFERENCES CITED

Andre, Marc. 1932. Note sur un acarien (*Penthaleus major Dugés*) nuisible aux plantes potageres. Bull. Mus. Hist. Nat. **2**(5): 284–91.

Baker, E. W. 1946. New species of North and Central American mites of the family *Penthaleidae* (Acarina). Jour. Washington Acad. Sci. **36**(12): 421–5.

Baker, E. W., and G. W. Wharton. 1952. An introduction to acarology. 465 pp. Macmillan Co.

Banks, N. 1902. New genera and species of acarians. Canadian Ent. **34**: 172.

Banks, N. 1917. New mites, mostly economic (Arach. Acar.). Ent. News **28**: 193–9.

Campbell, Roy E. 1941. Further notes on the blue oat or pea mite, *Penthaleus major* (Dugés), in California. California Dept. Agric. Bull. **30**(3): 312–4.

Essig, E. O. 1939. The blue oat or pea mite, *Penthaleus major* (Dugés), in California. California Dept. Agric. Bull. **28**(10): 507–8.

Froggatt, W. W. 1921. The blue oat mite (*Notophallus bicolor*). Agric. Gaz. N. South Wales **32**(1): 33–4.

Marchal, P. 1908. Sur le *Notophallus haematopus* Koch nuisible aux cultures du pois (*Pisum sativum* L.) dans le midi de la France (Acar.) Soc. Ent. de France Bull. **3**: 41–42.

Middlekauff, W. W., and A. E. Pritchard. 1949. Field tests to control the mite, *Penthaleus major*. Jour. Econ. Ent. **42**(5): 852.

Swan, D. C. 1934. The blue oat mite (*Penthaleus major* Dugés). Jour. Agric. S. Australia **38**: 365–7.

Wildermuth, V. L. 1916. California green lacewing fly. Jour. Agric. Res. **6**(14): 517.

Womersley, H. 1933. On some Acarina from Australia and South Africa. Trans. Roy. Soc. S. Australia **57**: 108–12.

Womersley, H. 1935. On the name of the "blue oat mite" of Australia. Bull. Ent. Res. **26**: 163.

Womersley, H. 1941. The red-legged earth-mite (Acarina, Penthaleidae) of Australia. Trans. Roy. Soc. S. Australia **65**: 292–4.

BULLETIN OF THE ENTOMOLOGICAL SOCIETY OF AMERICA

Members should read this publication carefully! It sometimes contains announcements, notices, etc., not sent to the membership in any other way.

Further Study of Spray Combinations for Control of Apple Pests in Connecticut[1]

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Two years ago I reported to the Eastern Branch results of studies of several combinations of sprays for apples. In that report it appeared that not all the most effective combinations as far as pest control was concerned gave the best quality. Since then the complexities of the problem seem to have increased rather than decreased. To them has been added recently concern over residues so that to obtain complete insect control without spoiling flavor, burning the leaves, decreasing yields or exceeding residue tolerances has become a good deal like spitting into the wind.

Many of the modern insecticides are specific for one pest alone, but in controlling specific pests we are apt just to trade one problem for another unless we are careful to recognize the limitations of each material and provide antidotes in the form of additional materials. It is well known that problems existed with the older pesticides, such aphids and leafhoppers with arsenicals, rough fruit and decreased yields with sulfurs, blotching with late dithiocarbamate sprays. More recently tests with DDT and other chlorinated hydrocarbons showed them outstanding in their effects on increased mite abundance so that whenever codling moths are controlled with DDT, curculios with methoxychlor, or red-banded leaf-rollers with TDE we may find ourselves worse off than before unless measures are taken to control mites. *So* we add acaricides. Then we meet the problem of compatibilities —leaf burn if compatibilities are poor, reduced quality (poor flavor and color) if the effects are insidious or not easily discerned, possibly even reduced yields if the spray is not good. If we are lucky we get insect control for a few years until resistance develops. Then we start looking for new chemicals and start all over again.

As an example, several years' cooperative effort among northeastern technical men produced a general purpose mix which appeared to be satisfactory in most instances. Commercial mixes, however, have given trouble at least in Connecticut and Rhode Island, severe leaf spotting on apples and yellowing and drop of leaves of peaches. More caution appears to have been indicated here, or at least more study in those areas where injury occurred. Attempts to improve certain combinations of sprays are described below based partly on experience in Connecticut.

LEAD ARSENATE-FUNGICIDE COMBINATIONS.—*Lead arsenate, sulfur and/or ferbam.*—Deficiencies of this combination were notably lack of leafhopper, aphid or mite control and moderate to fair curculio control. Sawfly control was not needed but is now important. To bolster the curculio deficiency, methoxychlor was substituted. This was a definite improvement for curculio and destroyed leafhoppers, but it did not affect mites, which in fact increased instead of decreasing. Apple maggot control has not been helped especially by either methoxychlor or dieldrin which came into use later. Substitution of dieldrin in our 1955 programs allowed more codling moth to develop and there appears to be some slight evidence that scale is increasing in these plots. Substitution of captan or glyodin for the fungicides in this combination helped control russetting in some cases, but glyodin-lead arsenate has given trouble that has not been completely corrected with ferric sulfate, use of which was suggested by the manufacturer. So we stand about where we did before.

CHLORINATED HYDROCARBON COMBINATIONS.—The general purpose mix containing methoxychlor, captan and malathion, has already been mentioned. In 1955 in order to learn more about the action of the several ingredients as well as their effects on quality, we applied methoxychlor with captan, and malathion with captan in several plots. Results are interesting in showing a remarkable upsurge of mites in midseason with methoyxchlor-captan —the same as was observed in 1953 with methoxychlor, TDE, and captan. This condition furthermore was not corrected by TEPP in 1955 as easily as it was where other combinations were used, indicating much greater pressure from the mite population. Leaves turned brown and appeared to be burned easily by later applications. It would seem from this that all combinations of methoxychlor and captan or similar ones will need supplements for mites wherever used. Still other chlorinated hydrocarbons could be substituted but would have the same disadvantages together with greater difficulty in meeting tolerances especially if used in mid-summer. So we began to look in other directions for a suitable spray combination.

NON-TOXIC SPRAYS OR SPRAYS WITH MOSTLY UNLIMITED TOLERANCE.—Interest during 1954 and 1955 centered around ryania plus supplements. However, we are fast getting as involved here as with lead arsenate combinations. Substitution of lead arsenate in the early sprays and omitting them later, eliminated some of the poor control of curculio, but apple maggot control was very poor in 1954 and not completely satisfactory with added rotenone in 1955. In the 1955 tests at the University of Connecticut under the direction of W. D. Tunis, split schedules did prove reasonably satisfactory, and mite and codling moth control was good at Mt. Carmel in spite of a bad year for both in 1955. Quality has also remained good. The main deficiency in our tests has been lack of curculio control which could be counteracted by use of early sprays of lead arsenate or methoxychlor, as it was at the University of Connecticut. But we may easily begin to go 'round and 'round again the same as with the lead arsenate combinations and substitutes. However, continued study of non-toxic chemicals should not necessarily be abandoned in the Northeast. Foliage quality and fruit flavor are reasons important enough, even though total controls may be low.

THE FUNGICIDES.—Effects of fungicides on yields, certain insect populations and flavor has been noted from time to time. Growers are constantly trying to improve

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quality of finish and color and have jumped at the use of captan and glyodin because of favorable characteristics. Like the insecticides, however, defects do not show the first year or for several years. It is believed that nitrogen-containing fungicides in general are beneficial, but we are not so sure of those containing chlorine. The phenomenal kick-back from mites with combinations of captan and chlorinated hydrocarbons suggests a possible role of excess chlorine since both methoxychlor and captan contain considerable amounts of chlorine. On the other hand, while control of scab is good with captan and glyodin, increase of mildew with both is important and it may well be necessary to start again at the beginning with sulfur. At any rate, great caution is needed in research and development in order not to go too far astray with newer chemicals. The need for more and more study of the actions and interactions of proposed combinations on the biological complex of the orchard is certainly indicated. Perhaps we need to approach the whole from a completely different angle.

RESULTS.—General results from our work with various spray combinations during 1952–55 inclusive are briefly given below. Lack of space does not permit giving details of individual tests.

COMBINATIONS INVESTIGATED

1952

1) Lead arsenate-sulfur-ferbam
2) Lead arsenate-glyodin
3) Lead arsenate-captan
4) Lead arsenate-dichlone
5) 1189[a]-sulfur-ferbam
6) Methoxychlor-TDE-sulfur-ferbam
7) Methoxychlor-nabam
8) Check—sulfur-ferbam

1953

1) Lead arsenate-captan
2) Lead arsenate-captan-glyodin
3) Dilan-TDE-ferbam
4) Dilan-TDE-captan
5) Methoxychlor-TDE-captan
6) Methoxychlor-TDE-maneb
7) DDT-parathion-dichlone (early), ferbam (late)
8) Check—no spray

1954

1) Lead arsenate-captan-malathion
2) Methoxychlor-DDT-captan-malathion
3) Methoxychlor-DDT-captan-glyodin
4) Parathion-nabam-ferric sulfate
5) DDT-parathion-glyodin
6) Ryania-glyodin-malathion
7) Diazinon-Lo-738[b]
8) Check—no spray

1955

1) Lead arsenate-captan
2) Dieldrin (early)-lead arsenate-captan
3) Malathion-captan
4) Diazinon-captan-glyodin
5) Methoxychlor-captan
6) DDT-parathion-captan-glyodin
7) Ryania-captan-glyodin, plus rotenone in late season
8) Check—captan only

[a] A product of the General Chemical Co. with the following formula: 2,3,3a,4,5,6,7,7a,8,8-Decachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene-1-one.
[b] Ethylene bis thiuran trisulfide, made by Rohm & Haas Co.

PLUM CURCULIO (*Conotrachelus nenuphar* (Hbst.)).—Best control continues to be obtained with methoxychlor. Dieldrin in early sprays was no better, though somewhat cheaper.

MITES (*Tetranychus telarius* (L.) and *Metatetranychus ulmi* (Koch)).—With mixtures used in 1952 and 1953 it was necessary to apply acaricides in midsummer. In 1954 acaricides were not needed other than what was used in the several mixtures. In 1955, no acaricides were needed on combinations numbered 3, 4, 6, and 7, but mites became severe on No. 5. Acaricides were used on Nos. 1 and 2. Ryania-glyodin plots had no mite trouble either in 1954 or 1955.

CODLING MOTH (*Carpocapsa pomonella* (L.)).—Presented no problems until 1955 when a severe infestation occurred. Controls were best with chlorinated hydrocarbons the same as noted in 1952 and 1953. Ryania continued to give promising results both in 1954 and 1955.

APPLE SCAB continued to be better controlled with captan in 1954, but so little was present in 1955 that the results mean nothing.

APPLE APHIDS (*Anuraphis roseus* Baker, *Aphis pomi* DeG., and *Eriosoma lanigerum* (Hausm.)) were less abundant in 1952 and 1953 with chlorinated hydrocarbons, while DDT-parathion showed improvements over all others. In 1954, rosy apple aphids were severe in all lead arsenate plots even though malathion was added. Wooly apple aphid, however, became more abundant in the methoxychlor-TDE-captan-glyodin plot than any other. In 1955 best control appeared to be with malathion-captan and DDT-parathion-captan-glyodin. From this it would appear that the phosphates controlled aphids fairly well except when combined with lead arsenate.

EUROPEAN APPLE SAWFLY (*Hoplocampa testudinea* (Klug)).—The best control up to 1953 was obtained with chlorinated hydrocarbons and DDT-parathion. This was equalled in 1955 with applications of dieldrin in early sprays to calyx using lead arsenate thereafter.

WHITE APPLE LEAFHOPPER (*Typhlocyba pomaria* McA.).—Leafhoppers have not been a problem since 1953 and therefore no data were taken in 1954 and 1955. From the earlier tests it was evident that chlorinated hydrocarbons and phosphates gave good control.

POWDERY MILDEW began to show in plots in 1954 and continued to increase in 1955 presumably because of extensive use of captan and glyodin. It was most abundant in trees sprayed with methoxychlor-captan and least abundant with DDT-parathion-captan-glyodin. Reasons for the low incidence in the latter case are obscure.

FRUIT FINISH was outstanding in 1953 with lead arsenate-captan or methoxychlor-captan. In 1955 extensive russetting on many varieties resulted with all treatments which may have been associated with extreme heat for this locality. Color appeared to be improved in the malathion-captan plots over lead arsenate-captan, though about equal in the ryania-captan-glyodin plots. Evidently lead arsenate slowed down coloration of MacIntosh the same as observed in previous years.

SCALD.—Very little scald has been observed in any plot since the elimination of sulfur in 1952.

TOTAL CLEAN FRUIT (External examination).—This

Table 1.—Percentage of apples free of external insect and disease blemishes. 1954.

	Gravenstein		McIntosh	
	Windfall	Picked	Windfall	Picked
Methoxychlor-TDE-captan-malathion	92.9	95.2	95.8	94.1
Methoxychlor-TDE-captan-glyodin	94.0	89.3	93.8	94.2
Lead arsenate-captan-malathion	87.7	76.5	93.4	87.0
Ryania-glyodin-(malathion to calyx)	54.1	64.0	80.5	80.9
Parathion-nabam-ferric sulfate	67.7	65.1	72.0	64.0
DDT-parathion-glyodin	88.8	88.3	89.4	84.6

seems to continue to favor such combinations as methoxychlor-TDE-captan and malathion in 1954, and methoxychlor-captan in 1955. Lack of an acaricide as noted in 1955 affected size.

LEAF SPOTTING was estimated in 1955 and appeared to be less abundant where dieldrin was applied to calyx together with captan. It was highest with Diazinon-captan and malathion-captan, indicating the influence of phosphates on this phenomenon.

FLAVOR.—Continued efforts to evaluate flavor in 1954 and 1955 seem to indicate superiority in the 1954 results for ryania-glyodin (with malathion early) over lead arsenate-captan-malathion throughout the season. However, canned sauce did not seem to show any differences amongst any of the treatments. The preference for frozen sauces made from apples treated with lead arsenate-captan-malathion over those treated with DDT-parathion-captan indicated possibly slight injury from the parathion.

COSTS.—The costs of the various combinations were estimated in 1954 and 1955. They indicated a very low cost for nabam-parathion and next in line are DDT-parathion-captan or captan-glyodin mixtures. High figures were obtained for methoxychlor-TDE-captan-malathion mixtures. Of the 1955 series the lowest costs seem to center on lead arsenate mixtures.

Table 2.—Percentage of apples free of external insect and disease blemishes. 1955.

Materials	McIntosh	
	Drops (July 1)	Harvested (Sept. 15)
Methoxychlor-captan	90.3	96.2[a]
Dieldrin-lead arsenate-captan	93.5	64.8
DDT-parathion-captan-glyodin	81.4	83.2
Methoxychlor-malathion	76.9	78.8
Ryania-captan-glyodin	72.0	50.8
Lead arsenate-captan	59.5	76.9
Diazinon-captan-glyodin	38.1	31.9

[a] Severe mite damage.

SUMMARY.—Selection of spray combinations involves study of many insects and diseases, as well as color, flavor and yields, which are affected indirectly by leaf damage. Our best mixtures were those used in 1954 as indicated by results given in tables 1 and 2. Non-toxic combinations need more study in the Northeast to avoid excessive plum curculio and apple maggot damage and to avoid the numerous substitutions which became necessary with lead arsenate and chlorinated hydrocarbons. Study of these, however, should not be abandoned.

Comparative Effect of DDT-Treated Houses on Introduced *Anopheles quadrimaculatus* Adults, 1944 and 1955[1]

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In 1944, the Tennessee Valley Authority sprayed 67 houses with DDT to obtain more information on the residual effect of these sprays against adults of *Anopheles quadrimaculatus* Say when applied under practical routine field conditions. Also, more detailed information under controlled conditions was obtained from one experimental house. A small, one-room, unoccupied house near Blackwell Swamp, Wheeler Reservoir, was mosquito-proofed, papered, fitted with two window traps, and sprayed with a 5% DDT-xylene-Triton mixture at the rate of approximately 250 mg. DDT per square foot. For each test natural populations of *A. quadrimaculatus* mosquitoes were picked up from the edge of the swamp in nail kegs and released in this house at various intervals from July to October. Detailed observations as to their reactions to this exposure to DDT were made and recorded (Metcalf *et al.* 1945). This natural population of wild mosquitoes collected in this manner on the edge of their breeding grounds in diurnal shelters was usually composed of about twice as many males as females and the females were mostly unfed.

Since interest in mosquitoes developing resistance to DDT is world-wide, efforts were made in 1955 to duplicate some of the 1944 experimental house tests to determine whether or not, after 12 years' exposure to DDT as house sprays and in other forms, natural populations of adult *A. quadrimaculatus* still reacted to DDT exposure as they did in 1944.

Due to the nature of these tests and the many physical factors that could affect the experiment, exact duplication of any one test was never possible. Therefore, it was realized from the beginning that complete duplication of the 1944 tests could not be achieved in 1955.

CONDITIONS AND METHODS.—Since 1944, the extensive mosquito breeding grounds in the Wheeler Reservoir area around Blackwell Swamp have been virtually eliminated by TVA malaria control operations. However, natural populations of *A. quadrimaculatus* mosquitoes have persisted in considerable numbers on private land only 7 miles northwest from Blackwell Swamp where beavers have built dams across the creek, thus causing a 400-acre, uncleared impoundment which spreads out over the

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flat bottomland. This prolific mosquito breeding situation is surrounded by cotton fields and over 200 houses are located within the 2 mile zone. Although no larvicidal measures have been applied for mosquito control during the past 12 years, a considerable amount of DDT house spray and crop dust has been used. Since the area was highly endemic for malaria, all of the houses and premises within 1 mile of the breeding situation were treated with DDT for several years under the organized house spray programs sponsored by Federal, State, and County public health organizations. Through these programs the local population learned the value of DDT insecticides at an early date and have continued to use considerable amounts of DDT spray in their own homes even though the publicly financed programs were discontinued several years ago.

Numerous *Mansonia perturbans* are produced in this swamp as well as *A. quadrimaculatus*. Seven collections of biting mosquitoes during the summer of 1954 indicated that from the 2,000 mosquitoes collected biting people at night, 83.3% were *M. perturbans*. The bites of this vicious and aggressive mosquito have caused such great annoyance and discomfort to the human population during the summer months that it has literally "needled" most of them into using considerable amounts of DDT residual house sprays, aerosols, and space sprays, inside their houses each night, in order to get enough relief to permit sleep. Such regular and widespread use of DDT in this area doubtless contributed to the protection of humans from *A. quadrimaculatus* also. Although one barn harbored a maximum of 1,500 *A. quadrimaculatus* mosquitoes at the time of one inspection in 1954, fifty-nine houses within 1¼ mile of this breeding area were inspected during the same year and not a single mosquito was found in any of them.

The cotton fields surrounding this swamp have been frequently dusted during wet seasons for boll weevil with heavy concentrations of DDT, BHC, sulphur, and toxaphene. At such times all adult mosquitoes virtually disappear from the area or their numbers are reduced below the nuisance level. The effect of the cotton dusting operations on the mosquito larvae which are produced in the swamp and which might be affected by this DDT due to wind drift or surface water runoff is not known, but the adult mosquitoes in this area have had ample opportunities to come in contact with some form of DDT.

In 1955, in an effort to simulate the small experimental house used at Blackwell Swamp in 1944, a single room in a vacant tenant house near Beaverdam Swamp was mosquito-proofed, papered, fitted with a single window trap, and sprayed at the same rate and manner as was the house in 1944. The mosquitoes were collected for testing in nail kegs at the edge of the swamp in the same manner as before and they were essentially of the same composition, *i.e.*, two males to each female and the females were mostly unfed. In 1944 seven tests covered a period from 6 days to 87 days after spraying, while in 1955 five tests were made from 14 days to 40 days after spraying. After cotton dusting operations started in August, it was not possible to get enough wild mosquitoes for a test.

An untreated control house was not used in 1955. In 1944, each time mosquitoes were released in the "control house" or were permitted to enter at dawn, they found a dark corner and rested undisturbed throughout the period of each observation.

RESULTS.—Although the total number of days elapsing between spraying and testing were twice as many in 1944 as in 1955, within the limits of these tests, the number of days after spraying did not appear to be a limiting factor. In 1944, the time required for complete knockdown of the mosquitoes varied from 60 to 180 minutes, but this variation was thought to be due to temperature changes and differences in the inherent susceptibility of the mosquitoes rather than to a progressive decrease in the toxicity of the DDT-treated surface (Metcalf *et al.* 1945). The variation in time for complete knockdown inside the house in 1955 was not so great, being from 90 to 120 minutes. Also, for both years essentially the same pattern is noted in the length of time required for mosquitoes to leave the house (Table 1).

Of the total mosquitoes leaving the house in 1944, 90.6% were dead at the end of 4 hours and 99.0% were dead after 24 hours, while in 1955, 80.4% were dead at the end of 4 hours and 97.4% were dead at the end of 24 hours. Although some of the mosquitoes that were exposed to DDT in these house tests lived for as long as 28 days after exposure, the mortality data are not calculated beyond 24 hours because it is felt that as the length of time increases the reliability of mortality data decreases and that the deaths that occurred after 24 hours could be due to factors other than exposure to DDT.

DISCUSSION.—Due to the wide variations in the length of time required for complete knockdown or for the mosquitoes to leave the house in individual tests for both years, the data for the first 60 minutes of exposure time for all tests appear to be more valid for comparisons; therefore, the total tabulations of all tests for each year and the mean and standard deviation from the mean have not been plotted past 60 minutes (Table 1, Fig. 1).

In 1944, the rate of knockdown in 60 minutes varied from 26% to 47%, while the accumulative total for all tests (1–7) was 38%. The spread in 1955 was wider, bracketing the results of the 1944 tests, with 10 and 52%, respectively. The total knockdown in 60 minutes for all tests in 1955 (8–12) was 31%, 7% below the 1944 figure (Table 1).

From these data a difference is noted in the rates of egress from the treated room in 1944 and 1955. The outside limits for 60 minutes in 1944 were from 28% to 70% with a total for all tests (1–7) being 53%. In 1955, the range was from 21% to 38% with a total for all tests (8–12) being 29%. This difference between the two series of tests is not significant.

Figure 1 shows the mean and standard deviation from the mean in the per cent knockdown inside the houses and the per cent leaving the houses during the first 60-minute exposure time for both years. A slight variation in knockdown is noted with the standard deviation being greater in 1955 than in 1944. In 1944 the rate of exit was greater and the standard deviation wider than it was in 1955. The wide variation between individual tests causes the differences between mean values to be of no significance.

The differences in the per cent mortality of those leaving the house are not significant and are believed to be due to individual or physical variations. It should be re-

Table 1.—Effect of DDT-treated house on introduced adults of *Anopheles quadrimaculatus*. 1944 and 1955.

Test No.	Date	Days After Spraying	Time of Day	Temperature °F.	Total Number Mosquitoes Released	Per Cent of Total Mosquitoes Released That Were Knocked Down Inside House[a] — Time Lapse from Start of Experiment (15-minute periods)													Per Cent Mortality of Those Leaving House at End of	
						15	30	45	60	75	90	105	120	135	150	165	180	195	4 Hrs.	24 Hrs.
1	7- 8-44	6	6:50 A.M.	71	1584	9(8)	19(33)	29(52)	39(59)	40(60)									85.0	99.5
2	7-12-44	10	12:15 P.M.	90	494	6(28)	13(60)	29(69)	30(70)										95.0	—
3	8- 3-44	32	11:00 A.M.	94	416	12(12)	23(24)	36(36)	47(48)	49			[b]						—	100.0
4	8-19-44	48	1:10 P.M.	93	540	2(8)	11(20)	28(29)	45(37)	46(38)	46(40)	47(42)	48(43)	48(45)	49(47)	49(48)	50(50)	[c]	96.7	100.0
5	8-30-44	59	12:00 noon	93	237	1(3)	1(31)	7(38)	26(59)	35(61)	37	38	38	38	39				93.8	100.0
6	9- 6-44	66	1:35 P.M.	90	158	2(3)	11(9)	18(18)	43(28)	56(32)	60(37)	—(39)	—(40)						88.9	98.4
7	9-27-44	87	11:15 A.M.	87	460	3(18)	6(25)	14(34)	29(40)	36(43)	42(46)	42(51)	43(57)						95.4	100.0
Total Tests 1–7		1944			3889	6(10)	15(32)	26(45)	38(53)										90.6	99.0
					1944 Mean	5(11)	12(29)	23(39)	37(49)											
8	7-15-55	14	12:45 P.M.	80	177	1(1)	2(11)	4(18)	11(21)	30(32)	35(37)	43(46)	48(52)						72.8	100.0
9	7-27-55	26	12:45 P.M.	90	301	1(6)	8(12)	31(25)	52(30)	57(36)	59(41)								83.7	99.2
10	8- 3-55	33	12:00 noon	85	206	1(8)	6(15)	19(24)	38(34)	47(41)	52(48)								77.8	100.0
11	8- 5-55	35	6:30 A.M.	78	194	0(3)	0(6)	4(15)	10(23)	25(31)	34(39)	45(46)	48(52)						86.0	97.0
12	8-10-55	40	8:15 A.M.	80	104	0(2)	2(10)	6(14)	27(38)	38(43)	47(53)								80.0	85.4
Total Tests 8-12		1955			982	1(4)	4(11)	16(20)	31(29)										80.4	97.4
					1955 Mean	1(4)	4(11)	13(19)	28(29)											

[a] Figures in parentheses are per cent of total mosquitoes released that left house.
[b] 3% were not knocked down after 120 minutes exposure to DDT.
[c] 1 mosquito was not knocked down after 180 minutes exposure to DDT.

membered that the mosquitoes which escaped to the outside cone were exposed to the DDT-treated surfaces for varying lengths of time; however, it is doubtful that this can be correlated with the per cent mortality because in some instances the mosquitoes that were *not* knocked down inside the house after 2 and 3 hours of known exposure to DDT continued to live longer than all others that escaped or were knocked down.

CONCLUSIONS.—Results from both years' experiments show wide variation and individual differences in time required for complete knockdown in each test and the rate of egress from the DDT-treated rooms, but these differences are not significant, and, in general, the data from these limited observations indicate that the behavior pattern and reaction of adult *A. quadrimaculatus* mosquitoes to DDT-treated surfaces remain essentially the same as

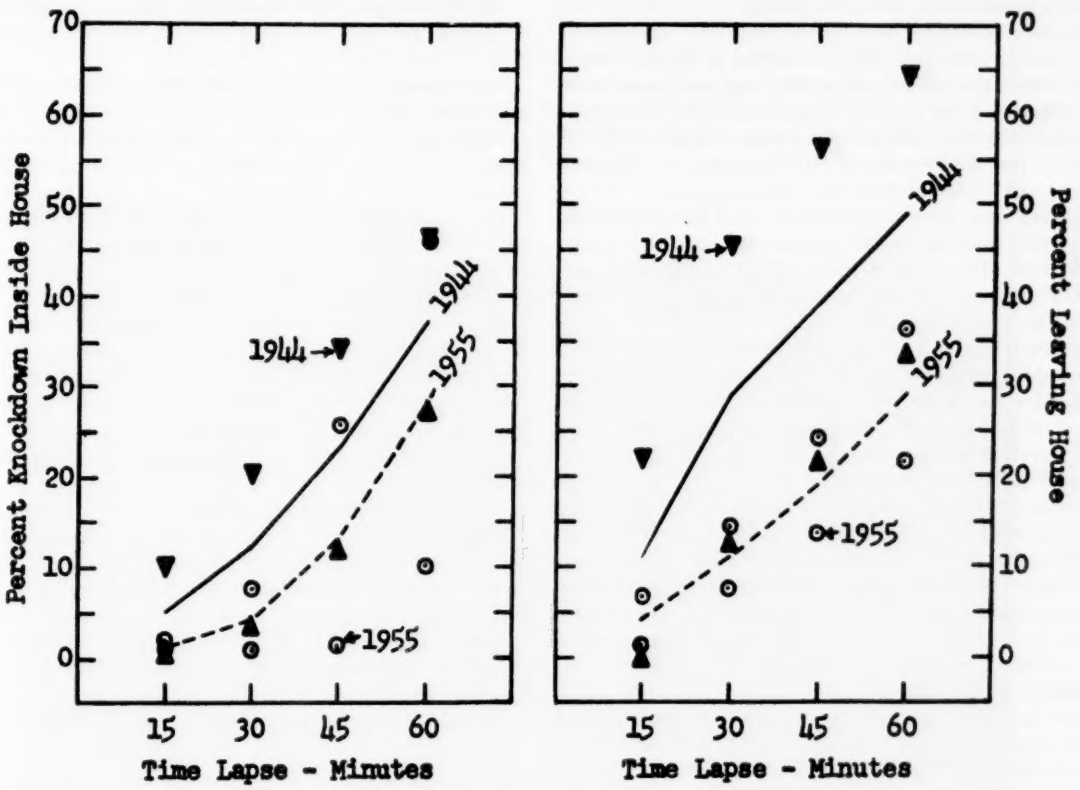

FIG. 1.—Comparative reactions of adult *Anopheles quadrimaculatus* to DDT-treated surfaces during the first 60 minutes after release showing the mean and the standard deviation from the mean. 1944 & 1955.

in 1944 after their natural exposure for 12 years to an area where DDT and related insecticides have been used.

ACKNOWLEDGMENTS.—It is a pleasure to acknowledge the assistance of all members of the Biology Section with this study. Special thanks are due Drs. O. M. Derryberry and F. E. Gartrell and Messrs. G. S. Christopher, W. B. Hawkins, and John B. Moore for their help and suggestions in analyzing the data and in preparing the final manuscript.

REFERENCE CITED

Metcalf, R. L., A. D. Hess, G. E. Smith, G. M. Jeffery, and G. L. Ludwig. 1954. Observations on the use of DDT for the control of *Anopheles quadrimaculatus*. Publ. Health Repts. 60(27): 753–74.

Experiments on Control of the Onion Thrips in Idaho[1]

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This is a final report of investigations for the control of the onion thrips (*Thrips tabaci* Lind.) conducted in the vicinity of Twin Falls, Idaho, from 1945 to 1952. Beginning in 1948, it was difficult to show increases in yields, especially in Sweet Spanish onions which had been shown by Sleesman (1943) to be less subject to heavy thrips infestations than other onion varieties. Horsfall & Fenton (1922), Wilcox & Howland (1950), and others have shown that flight of thrips from alfalfa, clover, peas, and other crops where they also breed is an important factor leading to outbreaks of this insect in onion fields. It may be that the widespread use of the new insecticides on other crops has had a tendency to reduce the seriousness of thrips as a pest to onions in the Twin Falls area.

In a previous report covering some of the experiments conducted in 1946 and 1947 (Douglass & Shirck 1949), it was shown that DDT, chlordane, and toxaphene were more effective when applied in emulsions than in suspensions and that the addition of nicotine sulfate or HETP improved the effectiveness of DDT suspensions. Mayeux & Wene (1950) found that low-volume sprays of 5 to 8 gallons per acre were effective and that less insecticide was needed than where 100 gallons were applied. Bronson & Rust (1951) found that mist-blower applications of DDT or parathion in 10 gallons of spray per acre controlled the thrips on bulb onions.

The work reported herein includes further tests of several combinations of insecticides in sprays, trials of some insecticides that were introduced in 1947, and comparisons of different methods of application. Most of the work was done on bulb onions, although dusts and aerosols were tested on seed onions.

METHODS.—Since commercial formulations were used except in 1948, the emulsifiers and solvents are indicated for the laboratory-prepared formulations only. Treatments were replicated five or more times and, except in the aerosol experiment, were arranged in randomized blocks. In experiments with farmer cooperators plots of bulb onions were from 8 to 48 rows wide and extended the length of the fields.

Four types of sprayers were used. A conventional tractor sprayer (Fig. 1) was used in commercial fields to deliver sprays of 150 gallons per acre at 400 pounds' pressure. The spray pump was driven from the tractor power take-off. A duster was also mounted on the same tractor. A muslin trailer, or apron, was attached to reduce drifting when dusts were applied. A swath 8 rows wide was treated with this machine.

A second high-pressure sprayer equipped with its own engine was used on small experimental plots, which were 8 rows wide and 50 feet long. This sprayer was hauled in a pickup truck alongside the plots and delivered about 200 gallons per acre at 275 pounds' pressure. Three hollow-cone nozzles per row were used on each of these sprayers.

A gear-pump sprayer, mounted on a tractor and driven by the power take-off, was used to apply 25 or 50 gallons per acre at pressures of 22 or 34 pounds. Three fan-type nozzles per row were used with this machine.

The fourth sprayer was a turbine blower that delivered $9\frac{3}{4}$ gallons per acre, covering a swath 16 rows wide (Fig. 2).[2]

Windy weather interfered with the application of dusts and mists; hence, these applications were usually made between 4 and 7 A.M., when ground temperatures were cooler and the air was calmer than later in the day. The dilute sprays were applied later in the day. Samples for thrips counts on bulb onions consisted of 10 plants per plot. The method described by Shirck (1948) was used in all population counts.

DDT aerosol was applied to seed umbels at 10 locations along one row of a farmer's field. Paper bags were tied over the umbels just before treatment, and the aerosol was discharged from a "bomb" through a hole in each bag. The hole was then closed, and after 5 minutes the bag was removed. Populations were determined from one umbel and yields from four umbels per replicate at each location.

Dusts were applied to seed onions with a small, electrically driven duster. The dust was discharged through a hole in the muslin canopy covering the plot (Fig. 3). This canopy was moved from plot to plot during application. Each plot consisted of 4 rows 8 feet long and contained approximately 140 umbels. Thrips counts were made on five umbels per plot, and all the remaining umbels were harvested to determine yields.

SPRAYS AND DUSTS ON BULB ONIONS.—Table 1 gives

[1] Accepted for publication January 23, 1956. Published with the approval of the Director of the Idaho Agricultural Experiment Station as Research Paper No. 393.

[2] V. E. Romney, formerly entomologist with the U. S. Department of Agriculture, supervised the turbine-blower applications.

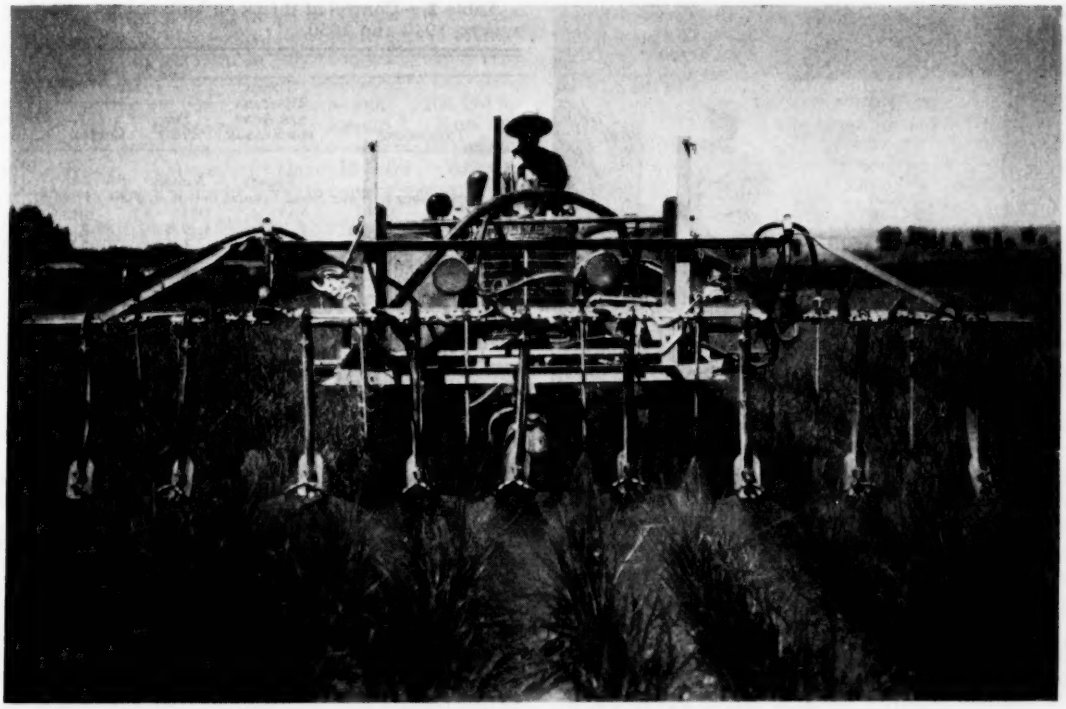

FIG. 1.—Sprayer-duster in use for control of onion thrips.

the results with various insecticides tested in high-volume sprays on bulb onions. There were six replications of treatments in 1948 and eight in 1949. The 1948 experiment was conducted in a commercial field at Filer, Idaho, and the tractor sprayer was used. In 1949 the applications were made with the truck-mounted sprayer to onions grown on the laboratory grounds. DDT emulsion gave significantly better control than DDT suspension sprays. The addition of nicotine sulfate to DDT suspension spray increased its effectiveness in 1948 but not in 1949. HETP and parathion also increased the effectiveness of DDT sprays, but TEPP did not. Under the light thrips infestations encountered in these experiments, none of the treatments gave a significant increase in yield.

FIG. 2.—Turbine blower that was used to treat onions.

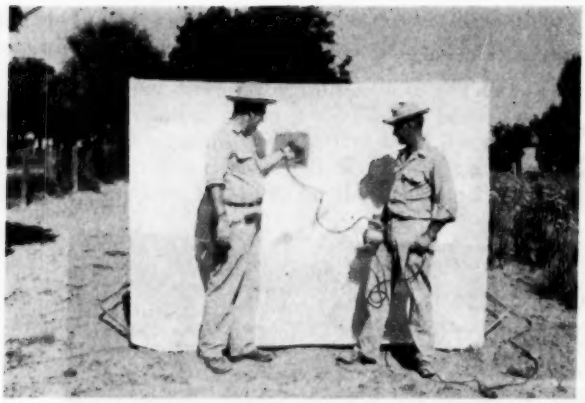

FIG. 3.—Applying dust to a plot of seed onions covered by a cloth canopy.

In 1949 and 1950 dusts were compared with sprays applied with the tractor machine on commercial bulb onions (Table 2). In 1949 chlordane and toxaphene sprays significantly increased the yields over the checks, but DDT did not in either a spray or a dust. In 1950 toxaphene both as a dust and a spray and a DDT dust gave significant increases in yield, but a DDT suspension spray did not. It was difficult to regulate the duster to deliver a predetermined quantity per acre, but it was concluded that dusts applied with ground equipment would give satisfactory control of thrips on onions grown for bulbs.

In 1951 high-volume applications of DDT with the tractor sprayer were compared with low-volume applications with the turbine blower on commercial bulb onions.

Table 2.—Control of thrips on bulb onions with dusts and sprays, 1949 and 1950.

TREATMENT	TOTAL TOXICANT PER ACRE FOR SEASON	THRIPS AFTER 4 DAYS		YIELD OF BULBS PER ACRE
		Per Plant	Control	
	Pounds	*Number*	*Per Cent*	*100-pound Bags*
Applications to White Sweet Spanish onions on June 13 and 21, 1949[a]				
Sprays:				
Toxaphene 50%, Em	3.4	0.8	88.3	411
Chlordane 45%, Em	2.8	1.5	79.5	391
DDT 25%, Em	2.8	.7	90.4	368
Dusts:				
DDT (8.85%)	5.0	1.1	85.2	374
Check	—	7.8	—	355
L.S.D. (19:1)		.6	n.s.	32
Applications July 17 and 20, 1950[b]				
Dusts:				
Toxaphene (10%)	3.0	0.6	84.5	521
DDT (10%)	3.2	1.6	74.5	520
Sprays:				
Toxaphene 50%, Em	1.8	.6	83.1	513
DDT 50%, WP	1.4	1.1	72.5	494
Check	—	6.4	—	461
L.S.D. (19:1)		2.2	n.s.	42

[a] 8 replications.
[b] 14 replications, 7 each on Yellow Sweet Spanish and Southport White Globe onions.

Treatments were replicated five times. Table 3 shows that better control and residual effect were obtained with the low-volume sprays. Yield differences were not significant, but the yield tended to be higher for the low-volume sprays and higher for the treated plots than for the checks. Incidental to this experiment, 1½ pounds of dieldrin and 2 pounds of toxaphene were also applied

Table 1.—Control of onion thrips on bulb onions with different insecticides applied in high-volume sprays.

FORMULATION (PER 100 GALLONS)	TOTAL TOXICANT PER ACRE FOR SEASON	THRIPS 4 DAYS AFTER APPLICATION		YIELD OF BULBS PER ACRE
		Per Plant	Control	
	Pounds	*Number*	*Per Cent*	*100-pound Bags*
Applications to Yellow Sweet Spanish onions on July 9 and 27, 1948				
DDT 50%, WP, 2 lb.; HETP 100%, 1 pt.; Triton X-155 3½ fl. oz.	DDT 3+HETP 3	1.0	91.5	626
DDT technical, 1 lb.; Velsicol AR-60, 3½ fl. oz., Triton X-155 3½ fl. oz.	DDT 3.1	1.1	90.7	659
DDT 50%, WP, 2 lb.; nicotine sulfate 40% 1 qt.; Triton X-155 3½ fl. oz.	DDT 3+nicotine sulfate 2.4	1.4	88.4	637
Tartar emetic, 4 lb.; granulated sugar, 4 lb.	Tartar emetic 12	2.3	81.4	656
DDT 50%, WP, 2 lb.; spreader sticker, ½ lb.	DDT 3.1	2.4	80.2	622
L.S.D. (19:1)	—	.8	—	n.s.
Check	—	12.1	—	623
L.S.D. (19:1)	—	—	7.1	n.s.
Applications to Southport Yellow Globe onions on July 11, 21, and 31, 1949				
DDT 50%, WP, 2 lb.; parathion 15%, WP, ½ lb.	DDT 6+parathion 0.5	0.5	90.7	258
Parathion 15%, WP, 1 lb.	Parathion .9	.7	88.0	275
DDT 25%, Em, 2½ qt.	DDT 5.7	.8	85.7	265
Toxaphene 50%, Em, 1½ qt.	Toxaphene 7.7	.9	84.9	291
DDT 50%, WP, 2 lb.; TEPP 40%, Em, ½ pt.	DDT 6+TEPP 1.2	1.1	80.2	222
DDT 50%, WP, 2 lb.; nicotine sulfate 40%, 1 qt.	DDT 6.4+nicotine sulfate 5.1	1.3	76.7	256
DDT 50%, WP, 2 lb.	DDT 5.8	1.3	76.0	261
Chlordane 45%, Em, 1¼ qt.	Chlordane 5.7	2.0	61.3	234
L.S.D. (19:1)	—	.5	—	n.s.
Check	—	6.9	—	235
L.S.D. (19:1)	—	1.1	10.3	n.s.

Table 3.—Control of onion thrips on Yellow Sweet Spanish bulb onions with high- and low-volume sprays of DDT applied at 1.5 pounds per acre on July 18 and August 3, 1951.

Kind of Spray	Gallons per Acre	Thrips per Plant After— 4 Days	15 Days	100-Pound Bags of Bulbs per Acre
High-volume	150	2.7	14.5	401
Low-volume	10	1.4	9.3	419
Untreated	—	9.7	20.2	376
L.S.D. (19:1)	—	4.2	n.s.	n.s.

with the turbine blower. There was a tendency for dieldrin to be more effective than toxaphene or DDT.

In 1952, $\frac{3}{4}$ pound of dieldrin was applied in 25, 50, 100 and 150 gallons per acre. The gear pump sprayer was used to apply the two lower volumes and the tractor sprayer to apply the higher ones. There were six replications of treatments. Table 4 shows that the volume of the spray did not affect the control of thrips or the yield of bulb onions.

Aerosols and Dusts on Seed Onions.—DDT aerosol applied to individually bagged onion umbels gave satisfactory control of thrips and a large increase in seed yields in 1945, as shown in table 5. Two applications were required to give a significant increase in yield.

Each year from 1947 through 1951 dust applications were made to small replicated plots of seed onions. Two applications were made in 1947, 1948, 1949, and 1951 and three in 1950. Twenty-five pounds per acre were used in 1947 and 1951 and 40 pounds in 1948, 1949, and 1950. Aldrin, BHC, chlordane, DDT, Dilan, lindane, nicotine sulfate, parathion, and toxaphene were used at various times during the 5 years. Mixtures containing DDT plus chlordane or nicotine sulfate were also tried. At least four different dusts were tested each year. All the materials except Dilan gave good control of thrips, but a significant increase in yield was not obtained in any season, apparently because of light thrips damage in the check plots. In 1947 and 1950 the yield was higher in the checks than

Table 4.—Control of thrips on White Sweet Spanish bulb onions with 3/4 pound of dieldrin applied in different volumes of spray, July 30, 1952.

Gallons per Acre	Thrips per Plant After— 4 Days	10 Days	Per Cent Control After 10 Days	100-Pound Bags of Bulbs per Acre
25	0.3	3.4	80.0	232
50	.1	3.4	80.1	218
100	.1	4.0	76.5	197
150	.3	3.5	79.3	227
Check	.6	17.0	—	206
L.S.D. (19:1)	0.3	4.8	n.s.	n.s.

Table 5.—Control of thrips on Yellow Sweet Spanish seed onions and increases in yields following treatments with DDT aerosol, 1945.[a]

Applications	Average Thrips per Umbel After 4 Days	Grams of Seed from 40 Umbels
Two (July 13, 24)	159	51.5
One (July 13)	117	27.3
Check	859	16.5
L.S.D. (19:1)	192	12.3

[a] Mean of 10 replicates.

in the treated plots, which indicated that the treatments may have had a harmful effect on pollinating insects.

Summary.—Various insecticides and methods of application were tested in southern Idaho for control of the onion thrips (*Thrips tabaci* Lind.) from 1945 to 1952. Dieldrin, heptachlor, parathion, and toxaphene were the most effective materials used. DDT gave better results when used as an emulsion rather than as a suspension spray. Addition of nicotine sulfate, HETP, or parathion to DDT suspension sprays improved their effectiveness, but such combinations did not seem justified under the low thrips infestations encountered. Low-volume sprays of $9\frac{3}{4}$ gallons per acre applied with a turbine blower or of 25 gallons per acre with a gear-pump sprayer were fully as effective as 100 to 150 gallons per acre with a tractor-power sprayer. DDT and toxaphene were effective applied as dusts, although more insecticide was needed than in the sprays. DDT aerosol applied to bagged seed umbels in 1945 controlled the thrips and gave a significant increase in yield. However, experiments for control of thrips on seed onions by applications of dusts did not show a significant increase in yield of onion seed in any of the 5 years when tests were conducted.

References Cited

Bronson, T. E., and R. E. Rust. 1951. Mist sprays for control of certain truck crop insects. Jour. Econ. Ent. **44**(2): 218–20.

Douglass, J. R., and F. H. Shirck. 1949. Experiments for control of onion thrips. Jour. Econ. Ent. **42**(1): 68–72.

Horsfall, J. L., and F. A. Fenton. 1922. Onion thrips in Iowa. Iowa Agric. Expt. Sta. Bull. **205**: 65–6.

Mayeux, Herman S., and George P. Wene. 1950. Control of onion thrips with low volume sprays. Jour. Econ. Ent. **43**(6): 907–12.

Sleesman, J. P. 1943. Variations in thrips populations on onions. Ohio Agric. Expt. Sta. Bimonthly Bull. **28**: 96–100.

Shirck, F. H. 1948. Collecting and counting onion thrips from samples of vegetation. Jour. Econ. Ent. **41**(1): 121–3.

Wilcox, J., and A. F. Howland. 1950. New insecticides for control of onion thrips in southern California. Jour. Econ. Ent. **43**(5): 690–3.

Flea Beetle Control on Irish Potatoes in Eastern Virginia[1]

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Flea beetles are important pests of the spring Irish potato crop on the Eastern Shore of Virginia. According to Anderson (1942), usually about 95% of the flea beetles are potato flea beetles, *Epitrix cucumeris* (Harr.); about 5% are tobacco flea beetles, *Epitrix hirtipennis* (Melsh.); and less than 1% are eggplant flea beetles, *Epitrix fuscula* Crotch. The western potato flea beetle, *Epitrix subcrinata* (Lec.), does not occur here. Anderson (1942) has reported the life history of the potato flea beetle as it occurs in Accomack County, Virginia. Briefly, the beetles overwinter in the adult stage, emerge from hibernation in April and move to the new potato plants. Early in May, tiny white eggs are laid in the soil at the base of the plant where the larvae develop and feed on the roots and tubers. The peak emergence of this new brood occurs around the middle of June and it is these beetles that cause practically all of the foliage damage.

As a result of extensive experiments extending over a 10-year period, Anderson (1942) recommended five to seven applications of a calcium arsenate-Bordeaux (2-4-6-50) spray. Although not as effective as the spray, a dust containing a calcium arsenate-monohydrated copper sulfate-hydrated lime (25-20-55) mixture was suggested where spraying was impracticable. Calcium arsenate formulations continued to be the accepted method of flea beetle control until the appearance of the chlorinated hydrocarbons following World War II.

Experiment in 1947.—The results of spray tests at Onley, Virginia, 1947, as presented in table 1, are typical of the high degree of flea beetle control obtained with DDT. The test materials were applied to replicated plots of the Irish Cobbler variety four rows wide and 200 feet long by a power sprayer operating at 250 pounds pressure. Treatments were made on May 20, June 2, 10 and 17. The relative degree of flea beetle control was determined by selecting five leaflets at random from each of five hills of potatoes, extracting a ½-square-inch leaf plug from each leaflet by means of a cork borer and counting the flea beetle punctures or scars. This method was used in evaluating subsequent flea beetle tests.

Table 1.—Tests with foliage sprays to control the potato flea beetle on Irish potatoes. Onley, Virginia. 1947.

Treatment[a]	Flea Beetle Scars per 25 (0.5 Sq. In.) Leaf Samples		Yield U. S. No. 1 Potatoes (100 Lb. Bags/A.)
	Number	Per Cent Reduction	
Calcium Arsenate, 4 lbs. in Bordeaux (8-8-100)	79	84.5	118
DDT, 50%, W.P., 2 lbs., in Bordeaux (8-8-100)	11	97.8	155
DDT, 50%, W.P., 2 lbs., +neutral copper 5 lbs.	11	97.8	145
DDT, 50%, W.P., 2 lbs.	14	97.3	164
Calcium arsenate, 4 lbs.	121	76.3	141
DDT, 50%, W.P., 2 lbs. +zineb, 2 lbs.	15	97.1	157
Untreated check	511	—	123
L.S.D. at 5% level	44		14.8

[a] Sprays applied May 20, June 2, 10 and 17.

The data in table 1 indicate that all treatments gave a highly significant reduction in flea beetle injury when compared with the check. The DDT wettable powders with approximately 98% reduction in damage were significantly better than calcium arsenate or calcium arsenate-Bordeaux. Because of the superior results obtained with DDT, the use of calcium arsenate was largely discontinued and it was even anticipated that the flea beetle might cease to be a major problem in potato production on the Eastern Shore of Virginia. However, by 1953, so many reports of unsatisfactory control with DDT were received that it was decided to reactivate the flea beetle project.

Experiments in 1954.—In 1954 a total of 17 spray formulations and 10 dusts were compared as foliage treatments in an attempt to devise better control measures. Applications were made May 20, May 27 and June 7 by means of a rotary hand duster or by a knapsack type sprayer operating at 40 pounds pressure. All plots were five rows wide by 30 feet long and were replicated four times in randomized blocks. Records were taken from the center row and the degree of flea beetle control evaluated in the manner previously described.

Unfortunately, the potatoes "gave up" early due to the extremely hot, dry weather and the tops were almost dead by the time peak flea beetle emergence occurred. As a result, flea beetle feeding was greatly curtailed and it was rather difficult to draw definite conclusions. However, some useful leads (Tables 2 and 3) were obtained which served as a basis for further tests. Table 2 shows that aldrin, dieldrin, Dilan, endrin and heptachlor gave significantly better flea beetle control than DDT when compared as sprays. Dust formulations containing 1½% dieldrin, 1½% Dilan, 2% endrin, 2½% aldrin, 2½% heptachlor, 1% impregnated plus 4% regular DDT, and 1% parathion plus 5% DDT were superior to a straight 5% DDT dust (Table 3). In general, the sprays were slightly better than the dusts and the materials showed the same order of effectiveness whether tested as a spray or dust.

Preliminary observations (Hofmaster 1955) definitely showed that soil treatments with aldrin or heptachlor as recommended for wireworm control might materially reduce flea beetle damage to the potato tubers and foliage. With this in mind, soil treatments were included with foliage treatments in 1955.

Experiments in 1955.—On March 3, 1955, sections of the test area were treated with aldrin and heptachlor at 2.5 pounds per acre and immediately disked. Unfortunately, a rather heavy rain occurred shortly thereafter and the potatoes (Cobblers) were not planted until

[1]Presented at the 27th Annual Meeting of the Eastern Branch of the Entomological Society of America held in Baltimore, Md., November 21–22, 1955. Contribution from the Entomology Department, Virginia Truck Experiment Station, Norfolk, Paper No. 125, Journal Series. Approved for publication January 13, 1956. Accepted for publication January 23, 1956.

Table 2.—Tests with foliage sprays to control the potato flea beetle on Irish potatoes. Onley, Virginia. 1954.

Treatment and Rate of Actual Toxicant per Acre[a]	Flea Beetle Scars per 25 (0.5 Sq. In.) Leaf Samples		Yield in 100-Lb. Bags per Acre	
	Number	Per Cent Reduction	Bags U.S. Number 1	Total Bags
Aldrin, 0.5 lb. E.	15.0	93.5	76	122
Calcium arsenate-Bordeaux 4-8-8	30.5	86.8	71	119
DDT, 0.5 lb. E.	47.8	80.0	83	128
DDT, 0.5 lb. E.+dieldrin, 0.25 lb. E.	10.8	95.3	83	131
DDT, 0.5 lb. E.+malathion, 0.5 lb. E.	35.5	84.6	78	124
DDT, 0.5 lb. E.+parathion, 0.15 lb. W.P.	24.8	89.3	98	139
DDT, 0.5 lb. E.+0.1 lb. rotenone	34.3	85.1	83	126
Diazinon, 0.5 lb. W.P.	58.0	74.9	83	133
Dieldrin, 0.25 lb. E.	24.5	89.4	82	133
Dieldrin, 0.5 lb. E.	3.8	98.4	86	127
Dilan, 0.25 lb. E.	24.3	89.5	80	125
Dilan, 0.5 lb. E.	15.5	93.3	84	123
Endrin, 0.25 lb. E.	19.3	91.6	91	143
Endrin, 0.5 lb. E.	10.0	95.7	79	123
Heptachlor, 0.5 lb. E.	16.3	92.9	79	127
Lead arsenate, 2 lbs.+spray lime, 8 lbs.	79.5	65.6	74	122
Parathion, 0.3 lb. W.P.	60.8	73.7	88	134
DDT dust, 5%, 35 lbs./A.	68.3	70.4	78	125
Check	230.8	—	79	127
L.S.D. at 5% level	23.6		n.s.	n.s.

[a] Treated May 20, May 27 and June 7. Sprays applied in 100 gallons of water per acre.

Table 3.—Tests with dusts to control the potato flea beetle on Irish potatoes. Onley, Virginia. 1954.

Treatment[a]	Average Pounds per Acre	Flea Beetle Scars per 25 (0.5 Sq. In.) Leaf Samples		Yield in 100-Lb. Bags per Acre	
		Number	Per Cent Reduction	Bags U. S. Number 1	Total Bags
Aldrin, 2.5%	36.1	20.8	90.8	94	143
DDT, 5%	38.7	57.3	73.6	79	130
DDT, 1% impregnated+4% regular	30.1	28.3	86.9	92	127
Diazinon, 4%	32.3	53.5	75.3	82	133
Dieldrin, 1.5%	33.2	27.3	87.4	89	136
Dilan, 1.5%	33.6	24.0	88.9	85	122
Endrin, 2%	32.0	14.5	93.3	92	139
Heptachlor, 2.5%	35.4	24.5	88.7	99	140
Lead arsenate, 2%+lime	32.5	60.8	72.0	79	118
Parathion, 1%+5% DDT	35.4	14.5	93.3	88	119
DDT emulsion	0.5	45.0	79.2	81	127
Check	—	216.8	—	83	125
L.S.D. at 5% level		22.8		n.s.	n.s.

[a] Treated May 20, May 27 and June 7.

March 17. These plots received no further insecticidal treatment except on May 23 at which time the entire area was treated with 1% parathion dust for Colorado potato beetle control. Since all plots were treated and at a time when the bulk of the flea beetle population was underground in the larval form, this procedure is not believed to have influenced results.

The soil treatments were compared with the preferred foliage treatment, dieldrin, and also with plots receiving regular foliage sprays of aldrin and heptachlor in addition to the soil insecticides. Foliage sprays were applied May 25, June 7, June 14 and June 21 by means of a knapsack sprayer operating at 40 pounds pressure. The treatments were replicated four times in plots 11 rows wide by 50 feet long.

The insecticides tested, rates and method of application, and results obtained are given in table 4. Briefly, compared with the check all treatments gave a highly significant reduction in foliage damage. Soil treatments with aldrin and heptachlor alone effectively reduced flea beetle activity to a point where foliage injury was negligible (Figures 1 and 2), being 94.2% lower than the check. Supplementing the soil treatments with four applications of aldrin or heptachlor at 0.5 pounds actual per acre per treatment decreased foliage damage 99.2% and 99.0%, respectively, an increase of only 5.0% and 4.7% over soil treatments alone. Four applications of dieldrin as a foliage spray at 0.2 pounds actual per acre per treatment did not give a significant reduction over soil treatment —97.8% as compared with 94.2%.

The soil treatments showed highly significant reductions in tuber damage as compared with the dieldrin foliage spray and the check. As might be expected, foliage sprays alone did not decrease tuber injury.

All treatments gave marked yield increases, ranging from 74.0% to 88.5% over the untreated checks. There were no significant differences between the insecticides or methods of application. Table 4 also indicates that

Table 4.—Tests with foliage sprays and soil treatments to control the potato flea beetle on Irish potatoes. Onley, Virginia. 1955.

Material	Method of Application	Rate/Acre (Lbs. Actual)	Flea Beetle Scars[a]			Yield in U. S. No. 1 Potatoes July 15		
			Foliage June 27		Tubers July 15		100-lb. Bags/A.	
			Number	Per Cent Reduction	Number	Number per Plot[b]	Number	Per Cent Increase
Aldrin	Soil	2.5	210	94.2	7	202	169	76.0
Heptachlor	Soil	2.5	210	94.2	4	213	181	88.5
Aldrin[c]	Soil+	2.5						
	Foliage	0.5	29	99.2	5	206	173	80.2
Heptachlor[c]	Soil+	2.5						
	Foliage	0.5	36	99.0	5	210	167	74.0
Dieldrin	Foliage	0.2	81	97.8	284	216	174	81.3
Check	—	—	3630	—	296	209	96	—
L.S.D. at 5% level			163		36	n.s.	18.4	

[a] Number of scars per 100 leaf samples (0.5 sq. in.) and per 100 square inches of tuber surface (one-square-inch area examined per tuber).
[b] Tubers counted in sub plots of 0.01 acre, taken at random from within the main plots.
[c] Soil treatments applied March 3. Foliage treatments applied May 25, June 7, 14 and 21 at rates indicated.

FIG. 1.—Potato flea beetle control through the use of soil insecticides. Foreground, untreated plot of Irish potatoes completely defoliated by flea beetles; background, treated with aldrin at 2.5 lbs. actual per acre; right, treated with heptachlor at 2.5 lbs. actual per acre.

there were no differences in the number of potatoes from any treatment; hence, the low yield in the check was necessarily due to a decrease in individual tuber size. This decrease in size is directly attributed to the fact that the plants in the untreated plots were almost completely defoliated by flea beetles some 3 weeks before reaching maturity.

In another series of tests, DDT, aldrin, Diazinon, dieldrin, endrin and heptachlor were compared as foliage sprays. All plots were 25 feet long by 5 rows wide and were replicated four times in randomized blocks. Application was by means of a hand-operated knapsack type sprayer operating at 40 pounds pressure.

The data in table 5 show that all insecticidal treat-

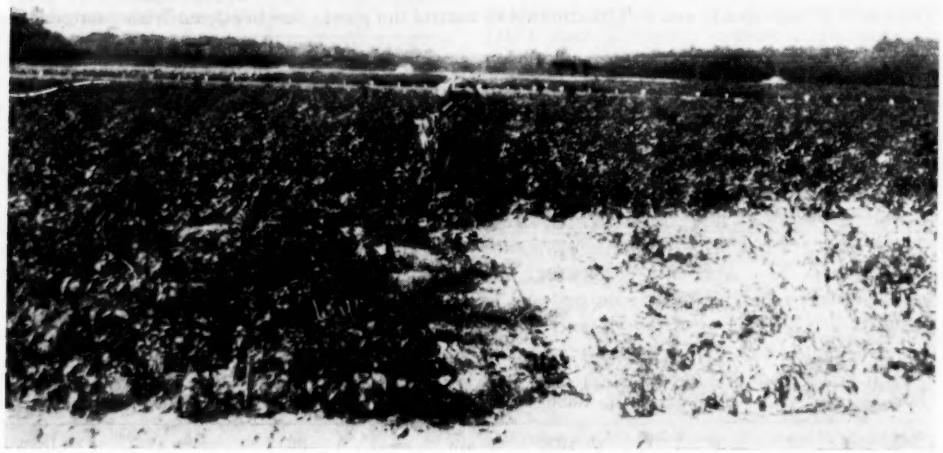

FIG. 2.—Lateral view of plots shown in figure 1. Left foreground, treated with aldrin at 2.5 lbs./A.; right foreground, untreated check; background, treated with heptachlor at 2.5 lbs./A. Note sharp break between treated and untreated areas.

Table 5.—Tests with foliage sprays to control the potato flea beetle on Irish potatoes. Onley, Virginia. 1955.

Treatment[a]	Rate/Acre (Lbs. Actual)	Flea Beetle Scars[b] June 27		Yield of Potatoes in 100 Lb. Bags per Acre July 15	
		Number	Per Cent Reduction	Number 1	Number 2
DDT	0.5	313	89.8	161	30
Heptachlor	0.5	163	94.7	160	29
Aldrin	0.5	160	94.8	159	19
Endrin	0.15	158	94.8	165	25
Endrin	0.2	83	97.3	174	25
Dieldrin	0.2	98	96.8	151	32
Diazinon[c]	1.0	230	92.5	167	27
Check	—	3058	—	104	26
L.S.D. 5%		293		38.4	n.s.

[a] Sprays applied May 25, June 7, 14 and 21, 1955 in 100 gallons of water per acre.
[b] Number of scars per 100 leaf samples (0.5 sq. inch).
[c] Applied as a wettable powder; all other materials applied as emulsions.

ments gave highly significant reductions in foliage damage and that there were no differences between insecticides. DDT appeared to give somewhat poorer control than the other materials but not significantly so. In visual scoring, foliage damage in the DDT-treated plots was consistently rated higher than any other insecticidal treatment. However, there were no yield differences between the insecticides and all were superior to the untreated check, giving increases of from 45.2% to 67.3% in U.S. No. 1 potatoes.

Discussion.—The importance of proper timing of foliage sprays for flea beetle control cannot be overemphasized. In 1955 the peak emergence of the spring brood was 7 to 10 days late. As a result some growers thought that flea beetles would not be numerous and stopped treating too early. These individuals and others not following a definite control program suffered very heavy flea beetle damage and the reductions in yield that resulted were estimated to be as high as 50%.

It is of interest to note that, regardless of the number of foliage applications, no cases of severe flea beetle foliage injury were observed in fields in which the soil had been treated for wireworms with aldrin or heptachlor. The increased flea beetle control through the application of soil insecticides would appear to justify treatment even though wireworms are not a severe problem. Points to be considered are: (1) The danger of overloading the soil with insecticidal residues; (2) The possibility of reduced rates of soil insecticides for flea beetle control alone; and (3) The period of protection given by a particular soil insecticide. Morrison & Crowell (1952) reported 99% control of tuber flea beetle larvae (*Epitrix tuberis* Gent.) after 3 years in soil treated with aldrin, chlordane, dieldrin and gamma isomer of BHC at 10 pounds actual per acre.

Although we were unable to demonstrate the degree of DDT resistance reported by Kring (1955), DDT has shown a tendency to give poorer flea beetle control than when it was first used. Another factor to consider in the choice of an insecticide for flea beetle control lies in the possibility that, since aldrin, dieldrin and heptachlor are also effective against wireworms, repeated foliage applications of these materials may leave sufficient residues in the soil to control wireworms without further treatment.

Summary and Conclusions.—The potato flea beetle has shown some evidence of becoming resistant to DDT on the Eastern Shore of Virginia but may still be adequately controlled with this material if a rigid spray or dust schedule is followed. Aldrin, Diazinon, dieldrin, Dilan, endrin and heptachlor are somewhat more effective than DDT as foliage sprays against flea beetles.

Soil treatments of aldrin or heptachlor alone at 2.5 pounds actual per acre gave a 94.2% reduction in foliage injury and practically eliminated tuber damage. Although the degree of control was somewhat lower than that obtained with four foliage applications of dieldrin at 0.2 pounds actual per acre per application, there were no significant differences in yield. Supplementing the soil treatments with four foliage sprays of aldrin or heptachlor at 0.5 pounds actual per acre per application increased the degree of control to approximately 99%, but here again was not reflected in the yield. The beneficial effects of soil treatments through increased flea beetle control are rather clearly indicated by yields of 169 and 181 bags (100-pound) of U. S. No. 1 potatoes per acre for aldrin and heptachlor, respectively, as compared with a yield of only 96 bags per acre in the untreated check.

References Cited

Anderson, Lauren D. 1942. A ten-year study of the life history and control of the potato flea beetle in eastern Virginia. Abstract of Doctoral Dissertation No. 38, Ohio State University Press, 1942, 14 pages.

Hofmaster, R. N. 1955. Flea beetle control on Irish potatoes. Veg. Growers' News (Norfolk, Virginia) 9(10): 1.

Kring, James B. 1955. Control of DDT-resistant potato flea beetles. Connecticut Agric. Expt. Sta. Circ. **193.**

Morrison, H. E., and H. H. Crowell. 1952. Soil insecticide studies in Oregon. Jour. Econ. Ent. **45**(6): 1002–10.

Evaluation of Parathion in California Red Scale and Yellow Scale Eradication Programs[1]

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In several citrus-producing areas in southern and central California, the intensive suppression or eradication of infestations of the California red scale, *Aonidiella aurantii* (Mask.), and of the yellow scale, *A. citrina* (Coq.), is undertaken as the most practical means of coping with these citrus pests. For programs of this type to be successful over a period of years, it is essential not only to organize entire areas of citrus plantings which are largely uninfested and reasonably remote from more generally infested areas into functional districts, but also to employ the most effective means of detecting infestations within the area, of eliminating infestations found, and of preventing reinfestation from internal or external sources.

A number of such eradication districts have been organized in central and southern California and most of them are still in operation. Two of the oldest are voluntary districts which were organized in Ventura County in 1922, one as the Ventura County Citrus Protective League and the other as the Fillmore Citrus Protective District. Together, these two organizations have more than 1,300 members, and their operations encompass over 28,000 acres of citrus plantings. Other active eradication districts, all of which are organized under the provisions of the California State Agricultural Code as legal districts, are currently operating in the Hemet and Coachella valleys of Riverside County, in the southern part of Tulare County, and in several other citrus-producing areas of the state.

In the operation of these various eradication districts, methods of detecting infestations of California red or yellow scale on citrus or other host plants and means by which the establishment of new infestations can be avoided have been largely standardized and are assiduously practiced. The actual eradication procedures have also been standardized within the individual district, but because of the high costs of the current eradication programs and the frequent recurrence of scale in treated areas, the need for improved methods of eradication has long been recognized.

The introduction of parathion in 1950 as a particularly effective treatment for control of California red and yellow scale insects suggested the possibility of utilizing this material as a means of increasing the efficiency of eradication programs. Accordingly, during the summer of 1951, field tests were established by the Department of Entomology of the University of California at Riverside in cooperation with the Ventura County Citrus Protective League and the Southern Tulare County Citrus Protective District to evaluate the possibilities of advantageously including parathion in the eradication programs of these two groups for control of the California red scale and of the yellow scale, respectively. The results of these field studies are summarized in this paper.

Experimental Procedures and Results.—The measures employed for the attempted eradication of scale insects on citrus have usually consisted of a series of closely sequenced treatments applied to the known infested trees and to all the trees within a specified area surrounding these infested trees. Fumigations with hydrocyanic acid (HCN) and spray applications of petroleum oil have been utilized as specific treatments, the choice, number, and sequence of treatments being determined by such factors as time of year that scale was found, extent of infestation, resistance of scale to HCN fumigation, and seasonal tree tolerance to a specific treatment. Since there may be some time lag in initiating a field eradication schedule, it has become customary, immediately after determining the extent of the infestation in an individual property, to apply a so-called spot treatment to the known infested trees, and to a guard row surrounding them.

The need for a sequence of additional treatments on these known infested trees, as well as on the trees in a suitable buffer area, is predicated on certain considerations, namely, that (1) no known treatment tolerated by the tree host will provide a complete kill of the California red scale or of the yellow scale, (2) inefficiencies in the application of treatments are likely to be involved, and (3) the presence of live scale may not have been detected on other trees within the immediate vicinity of the known infested trees. The adequacy of eradication schedules based on a sequence of HCN fumigation and petroleum oil spray treatments may be evaluated by determining the number of groves showing no reinfestation within a reasonably short period after such eradication treatments (3 years without reinfestation is usually accepted as sufficient evidence). Without compensating for the possibilities of new introductions, the records available indicate that eradication has been achieved in many groves and that in areas where the scale is largely nonresistant to HCN fumigation eradication has been conclusively achieved in over 60% of the groves treated.

This and other evidence of the sound principles involved in the eradication procedures currently employed suggested the advisability of evaluating parathion within the limitations of these basic premises by substituting it for one or more of the HCN fumigation or petroleum oil spray treatments. Additionally, certain further limitations based on the use of individual treatments were taken into account, and ultimately the selection of individual eradication schedules was predicated on the following considerations and limitations:

1. Eradication schedules were restricted to a total of three treatments applied in as close sequence as possible, preferably not more than 30 days apart. Against yellow scale several two-treatment schedules were used.

2. Only HCN fumigation (F), petroleum oil spray (O), and parathion spray (P) treatments were considered to be suitable for the tests. (Combinations of petroleum oil and parathion were not included in these initial trials

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because of incomplete information with regard to tree tolerance.)

3. Because of tree-tolerance factors, no eradication schedule included more than a single petroleum oil spray treatment.

4. All treatments were applied at the maximum dosage tolerated by the trees and with maximum provision for effective coverage.

5. Since it was considered possible that in practice eradication treatments might be applied to trees actually infested but not known to be infested, and located beyond the guard rows surrounding the known infested trees, no spot treatment was included in the test schedules.

6. Eradication schedules were tested during a period of the year not considered ideal for maximum results from individual treatments.

7. Eradication plots were located in groves heavily infested with scale but situated as remotely as possible from other infested host plants.

8. Evaluation of results was based on individual tree inspections of the type normally employed in the eradication districts.

The location of test plots within eradication areas would have been ideal with reference to remoteness of location from other infested host material, but this was impracticable from the standpoint both of availability of a suitable scale population and of jeopardy to the eradication program in the immediate area. On the other hand, the obvious limitations of attempting an appraisal of eradication programs in other areas suggested the advisability of making only preliminary tests. Single test sites were therefore established in a California red scale-infested grove in the Riverside-Arlington Heights area of Riverside County and in a yellow scale-infested grove at Ivanhoe, Tulare County.

Riverside County Tests with HCN-Resistant California Red Scale.—The test plot, which was located in the Riverside-Arlington Heights district was situated in a fully mature Valencia orange grove heavily infested with HCN-resistant California red scale. The plot area was bordered on the north by a citrus grove which had a light California red scale population, and which had been sprayed with parathion during the spring period preceding the start of the eradication test. The south and east borders were parts of the same grove, which were treated with parathion at the start of the test, with provision for another treatment during the next postbloom period. The western border was an open, uncultivated field. Although scale infestations in the bordering groves were maintained at low population levels, opportunities for reinfestation of the test trees existed.

The composition of the individual treatments and of the test eradication schedules is shown in table 1. Each eradication schedule was replicated five times with subplots consisting of single trees. Spray applications were made by project personnel by conventional methods designed to secure maximum coverage. The fumigation treatments were undertaken by a highly competent pest control operator in cooperation with project personnel. The third series of fumigation treatments was originally scheduled to be applied in September 1951, allowing a 4- to 6-week interval between eradication treatments; but because of the severe injury caused by the second fumigation, the final fumigation was postponed until the following winter period. Adverse tree reactions following the spray application of medium-grade petroleum oil in the first series of treatments necessitated the use of light-medium petroleum oil in the second and third series of treatments. The second series of spray treatments was delayed to some extent (indirectly because of the fumigation injury), and the third series of spray treatments was also postponed so as to approximate the winter fumigation date.

The first inspection following the completion of the eradication schedules was made on July 17 and 18, 1952. The final inspection was made on December 9 and 10, 1952. An experienced inspection crew of the Ventura County Citrus Protective League made both inspections. Forty-five minutes were allotted for the inspection of each tree, and ladders were used to facilitate the searching in the upper portions of the trees. At the time of the July, 1952, inspection, live California red scales were

Table 1.—Date of application, composition of treatment, and sequence of fumigation and spray treatments used in eradication schedules[a] against HCN-resistant California red scale in Riverside-Arlington Heights experimental plot.

	HCN FUMIGATION (F)		PETROLEUM OIL SPRAY (O)		PARATHION SPRAY (P)	
TREATMENT SEQUENCE	Date	Dosage, cc./Unit	Date	Dosage per 100 Gal.	Date	Dosage per 100 Gal.
First	7-31-51	18	8-16-51	Medium grade, emulsive type 2 gal.	8-16-51	2.5 lbs., WP, 25%
Second	8-30-51	18	10-12-51	Light-medium grade, emulsive type 2 gal.	10-12-51	Same
Third	2-19-52	20	2-27-52	Same	2-27-52	Same

[a] Eradication schedules indicating sequence of treatments:

FFF	OFF	PPP
FFO	OFP	PPF
FFP	OPP	PPO
FOF	OPF	PFF
FOP		PFO
FPP		PFP
FPF		POF
FPO		POP

found in four of the trees. These infested trees had been treated with the following schedules:[a] OPP, OPP, FOP, and PFO. All four trees were subsequently sprayed with parathion wettable powder at a dosage of 2½ pounds of 25% material per 100 gallons.

At the outset of the December inspection it became obvious that most of the trees were infested, and the decision was made to determine the number of localized infestations present in each tree (without regard to the number of scales actually present in each localized infestation) instead of following the earlier pattern of discontinuing the search as soon as a single live scale had been found on a tree. The results of the determinations made in this manner are summarized in table 2.

Tulare County Tests with Yellow Scale.—The test plot was situated within and to one side of a large planting of navel oranges at Ivanhoe, and was adjacent on the exposed side to a large grape vineyard. The trees were relatively young (about 10 feet high) and very vigorous and heavily foliaged. The yellow scale infestation was light but very uniform throughout the test area. A pre-treatment inspection indicated the presence of live yellow scale in every tree except one, and dead scales were found on this tree. The rest of the grove was fumigated at the time the test was started, with the result that possibilities of reinfestation from that area were greatly minimized.

Table 2.—Average number of localized infestations of live HCN-resistant California red scale in Riverside-Arlington Heights plot at time of December 10–11, 1952, inspection.[a]

Composition of Eradication Schedule	Average Number of Localized Infestations per Tree	
	Excluding Infested Trees of July Inspection	With Weighted Values for Infested Trees of July Inspection
First, Second, and Third Sequence Occurrence of Treatment[b]		
First treatment of schedules was		
Parathion (P)	5.3	5.6
Petroleum oil (O)	5.5	6.3
HC fumigation (F)	6.0	6.2
Second treatment of schedules was		
Parathion (P)	5.4	6.0
HCN fumigation (F)	5.8	6.0
Petroleum oil (O)	5.9	6.5
Third treatment of schedules was		
Parathion (P)	5.5	6.1
HCN fumigation (F)	5.6	5.6
Petroleum oil (O)	6.0	6.2
All Sequences Included		
FFF (1 sequence)	6.0	6.0
FFO (3 sequences)	7.1	7.1
FFP (3 sequences)	5.2	5.3
FOP (6 sequences)	5.4	6.1
PPP (1 sequence)	6.3	6.3
PPF (3 sequences)	5.1	5.1
PPO (3 sequences)	5.3	6.2

[a] At the time of the July 17–18, 1952 ,inspection, four trees were found to be infested. Inspection of the tree was discontinued as soon as a live scale was found, and the trees were subsequently treated with parathion. On the assumption that had these trees not been treated they would have been as heavily infested as the most heavily infested tree found during the December 10–11, 1952, inspection, these calculations are based on the probability that fifteen (15) localized scale infestations would have been found in each tree instead of the actual numbers found, namely, 0, 0, 2, and 3.

[b] Position of individual treatment in eradication schedules irrespective of other treatments in schedules.

The specific treatments and the composition of the individual eradication schedules are outlined in table 3. Six single-tree replicates were provided in the original layout of the experiment, but randomization into subblocks was not attempted because of operational difficulties that would have been involved in completing the several fumigations. All applications were made in the manner described above, although the fumigations were undertaken by a local pest control operator from the Ivanhoe area.

At the time of the second series of spray treatments applications of parathion to trees which had previously been sprayed with petroleum oil caused severe injury consisting of a heavy leaf and green fruit drop. Parathion applications to trees previously sprayed with parathion or fumigated caused no tree injury. As a consequence, no parathion was applied in the third sequence of treatments to trees previously injured or to trees sprayed with petroleum oil in the second sequence of treatments. This necessary revision eliminated the OPP schedule of treatments.

The first inspection of the trees after completion of the eradication schedules was made on July 24 and 25, and the final inspection on December 10 and 11, 1952. Since the trees were relatively small, the inspection period was limited to 15 minutes per tree and no ladders were required.

Only two trees were found to be infested with live yellow scale at the time of the July inspection. These trees had received the following sequences of treatments: OF and FFO. The inspection made on December 10 and 11, 1952, indicated that live yellow scale was present on 25% of the treated trees. The results of this inspection are summarized in table 4. Among the individual eradication schedules under test, only one, a parathion spray treatment followed by two HCN fumigation treatments, did not have one or more of its replicate trees infested.

Discussion.—It was conceded at the outset of these studies that completely critical results could not be obtained since the available experimental areas did not preclude the possibility that the trees involved in the tests might become reinfested from external sources after completion of the eradication treatments. It was further appreciated that the scale populations within the test areas exposed to ineffective eradication schedules would ultimately provide a source of reinfestation within the individual test area. On the other hand, it was impracticable to establish the desired tests in specific eradication districts since known infestations were not sufficiently extensive or localized to make the tests possible. Furthermore, the placing of experimental trials where they would jeopardize adjacent plantings was not consistent with the sound eradication policies in effect.

For these reasons the tests were established for the purpose of obtaining trend information which might ultimately prove useful in evaluating the probable utility

[a] Letters indicate sequence of treatments in schedules: petroleum oil spray (O), parathion spray (P), and HCN fumigation (F).

Table 3.—Date of application, composition of treatment, and sequence of fumigation and spray treatments used in eradication schedules[a] against yellow scale in Ivanhoe experimental plot.

Treatment Sequence	HCN Fumigation (F)		Petroleum Oil Spray (O)		Parathion Spray (P)	
	Date	Dosage cc./Unit	Date	Dosage per 100 Gal.	Date	Dosage per 100 Gal.
First	7-27-51	20	7-17-51	Medium grade, emulsive type 1.75 gal.	7-17-51	2 lbs., WP, 25%
Second	8-27-51	20	8-14-51	Same	8-14-51	Same
Third	9-24-51	20	9-20-51	Same	9-20-51	Same

[a] Eradication schedules indicating sequence of treatments:

FF	FFF	OFF	PPP
FO	FFO	OPP[b]	PPF
FP	FFP		PPO
OF	FPP		PFF
OP			PFO
PP			POF
PF			
PO			

[b] Third treatment of OPP schedules not applied because of previous tree injury.

of parathion in eradication schedules. Such information would necessarily have to be correlated with more extensive information concerning the use of parathion as a practical maintenance treatment for California red scale and yellow scale control, and the critical limitations concerning its usage in any specific instance.

Table 4.—Average per cent of trees infested with live yellow scale in Ivanhoe plot at time of December 10–11, 1952, inspection.

Composition of Eradication Schedule	Average Per Cent of Trees Infested
First, Second, and Third Sequence Occurrence of Treatment[a]	
First treatment of schedules was	
Parathion (P)	19.6
Petroleum oil (O)	28.1
HCN fumigation (F)	29.5
Second treatment of schedules was	
HCN fumigation (F)	19.3
Parathion (P)	28.1
Petroleum oil (O)	33.3
Third treatment of schedules was	
HCN fumigation (F)	16.7
Parathion (P)	27.8
Petroleum oil (O)	27.8
All Sequences Included	
PP (1 sequence)	16.7
FF (1 sequence)	16.7
FO (2 sequences)	23.1
FP (2 sequences)	25.0
PO (2 sequences)	36.0
FFP (2 sequences)	8.3
PPP (1 sequence)	16.7
FFF (1 sequence)	16.7
PPO (1 sequence)	16.7
PFO (2 sequences)	25.0
PPF (2 sequences)	33.3
FFO (2 sequences)	33.3

[a] Position of individual treatment in eradication schedules irrespective of other treatments in schedules.

In addition to the above-mentioned restrictions imposed upon the interpretation of the test results, a necessary change in the scheduling of treatments in the Riverside County test, occasioned by the instance of tree injury following the second fumigation, influenced the results of the test. The severe injury of the trees fumigated on August 30 was not predictable or readily explained since all operational factors were carefully checked, both by the commercial crew and by project personnel who were present at time of the treatment. The severity of the injury precluded the possibility of making further applications to the injured trees until the full extent of the damage could be determined and until the trees were refoliated. The delays thus involved postponed the application of the second sequence of petroleum oil and parathion spray applications almost a month past the date originally projected. As a further change, a light-medium-grade petroleum oil was substituted in both the second and the third sequence of applications, since the medium-grade petroleum oil used in the first application caused a heavier than normal leaf drop.

The influence that these various alterations in composition and timing of treatments had on the performance of individual eradication schedules is not known, but it is likely that the delay of all third-sequence applications until February 1952, had less effect than the delay of second-sequence spray applications on the relative performance of the eradication schedules. This is suggested by the tendency for the closely sequenced schedules (approximately 2 weeks apart) of a petroleum oil or parathion spray application followed by a second-sequence fumigation, to be superior to schedules based on a fumigation followed by a second-sequence spray application made more than 2 months later.

The finding of live scale on only four trees at the time of the July 1952, inspection allowed only a limited interpretation of results. Two of the trees had received the same sequence of treatments, i.e., petroleum oil spray followed by two parathion sprays, whereas the other two trees received each of the three treatments but in two different sequences. No trees which received two or three HCN fumigations, three parathion sprays, or

combinations of parathion sprays and HCN fumigations were found to be infested.

The very general distribution of scale throughout the test block at the termination of the test in December 1952, tends to suggest the inadequacy of the eradication schedules under test to cope with heavy and long-established California red scale populations. Had reinfestation been largely from populations outside the test area, it would have been reasonable to have expected either a higher incidence of reinfestation along the borders of the test block, particularly along those borders contiguous with other citrus plantings, or a scatter pattern of reinfestation with some trees heavily reinfested in comparison with others receiving the same eradication schedule.

In actuality, the information obtained by determining the number of localized infestations per tree at the time of the December 1952 inspection indicates that the number of infestations in the border rows very nearly approximates the average number per tree for the entire test block, exclusive of the four trees found infested earlier and subsequently treated with parathion. The number of localized infestations per tree ranged from 2 to 15. The infestations were reasonably remote from one another within an individual tree, and the distribution was sufficiently random to vitiate any correlations with prevailing wind direction, bird habits, and so forth. Such fragmentary evidence suggested the advisability of analyzing the accrued data for trend information, even though the relationship of eradication schedule to apparent performance may not be rigidly assumed.

Such analyses are summarized in table 2. The results are computed on two bases since it is debatable whether the trees found infested in July and subsequently sprayed with parathion should be excluded from the summarization of the terminal count in December, on the premise that other trees receiving the same eradication schedules would provide a more comparable evaluation of the treatment; or whether these trees should be assigned values consistent with the highest observed in the December inspection on any of the other trees in the test, on the premise that they would have been infested as heavily or more heavily in the absence of re-treatment. Regardless of method of computation, the interpretation of the data suggests similar trends. In no instance does a petroleum oil spray appear to be a preferred treatment, either in a matter of sequence or in an eradication schedule composition. A parathion spray would appear to be the most desirable treatment with which to initiate an eradication schedule, and would preferably be followed by two HCN fumigations, or an HCN fumigation and a parathion spray. In the latter instance the parathion spray should probably precede the HCN fumigation. The combination of parathion sprays and HCN fumigations appears to be more effective than the exclusive use of either, and to that extent the limited inclusion of parathion in eradication schedules would presumably be justified.

The use of a spot treatment on known infested trees in addition to the three eradication treatments, and a closer timing of treatments, should be totally advantageous. A spot treatment of the known infested trees and two subsequent eradication treatments would appear to be inadequate eradication procedure.

In the Ivanhoe test plot, all applications were completed within 70 days of the start of the test. The spray treatments were started 10 days earlier than the fumigation treatments, but subsequent treatments of each were applied at approximately 30-day intervals. Tree injury encountered where parathion sprays were applied to trees previously sprayed with petroleum oil necessitated the omission of the OPP eradication schedule but did not otherwise alter the test or cause any delays in treatment. The trends indicated in the Ivanhoe test on yellow scale show some limited parallels to the results in the California red scale test. The bordering trees of the test plot had the same incidence of reinfestation as the non-bordering trees. With only 25% of the total trees reinfested by December 1952, a random incidence of the yellow scale infested trees was observed. Petroleum oil spray was in no instance the preferred treatment, either with regard to sequence of treatment or for inclusion in an eradication schedule. Parathion spray was indicated as the preferred initial treatment in an eradication schedule, with HCN fumigation preferred as the second- and third-sequence treatment. While this trend in the data is suggested by an interpretation of the pooled results (see Table 4), it is further interesting that of the 20 eradication schedules included in the test only the trees receiving the PFF sequence of treatments were entirely free of reinfestations at the termination of the test. In both the Riverside County and Tulare County tests, an eradication schedule consisting of two HCN fumigations and a petroleum oil spray was notably inferior.

Of the 66 trees receiving an eradication schedule of two treatments, 18 of them or 27.3% were reinfested whereas of the same number of trees receiving an eradication schedule of three treatments, 15 or 22.7% were reinfested. While this difference is not in itself striking, the indicated trend of the results helps to suggest that the incidence of reinfestation may have been influenced primarily by the eradication test schedules.

The results of the Ivanhoe test on yellow scale would again suggest the feasibility of including parathion in an eradication schedule, particularly as a substitution for a petroleum oil spray and/or as a substitution for a single HCN fumigation.

SUMMARY.—Field tests were conducted to determine whether or not parathion might be incorporated in the sequence of California red and yellow scale eradication treatments to increase the effectiveness, to reduce possible phytotoxic effects, and to increase the flexibility of the program.

Results of the California red scale test at Riverside, Riverside County, indicated that in no instance does a petroleum oil spray appear to be a preferred treatment, either in a sequence of treatments or in an eradication schedule. A parathion spray appears to be the most desirable treatment with which to initiate an eradication schedule, and it would preferably be followed by two HCN fumigations or by a second parathion spray and an HCN fumigation, in that order. The combination of parathion sprays and HCN fumigations appears to be more effective than the exclusive use of either, and to

that extent the limited inclusion of parathion in eradication schedules would persumably be justified.

Results of the tests on yellow scale at Ivanhoe, Tulare County, show some limited parallels to the results in the California red scale test. Petroleum oil sprays were in no instance the preferred treatment, either in a sequence of treatments or in an eradication schedule. Parathion spray was indicated as the preferred initial treatment in an eradication schedule, with HCN fumigation treatments preferred as the second and third sequence treatments.

In both tests, an eradication schedule consisting of two HCN fumigations and a petroleum oil spray was notably inferior.

The results of the Riverside County test on California red scale and the Tulare County test on yellow scale would suggest the feasibility of including parathion in an eradication schedule, particularly as a substitution for a petroleum oil spray and/or as a substitution for a single HCN fumigation.

Control of Larvae Infesting Sweet Corn Ears[1]

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Ear-infesting insects cause losses in the production of sweet corn by direct injury, by adverse changes in the appearance of the product and, finally, by fragment contamination of processed corn. Several species may be involved, but the two more-important ones in southeastern United States, are the corn earworm, *Heliothis zea* (Boddie) and the dusky sap bettle, *Carpophilus lugubris* Murry.

The corn earworm has been the subject of numerous investigations over the years, but there was little progress in its control until Barber (1940, 1944) found that pyrethrins, or dichloroethyl ether, dissolved in mineral oil, were effective. These chemicals are injected into the silk tube with a modified pump-type oil can. The method is very effective, but the labor involved makes it quite costly.

More recently, Blanchard *et al.* (1949) reported that control could be obtained with DDT. The use of this material has led to the development of a somewhat-less-expensive treatment, in which a DDT-mineral oil spray is applied during the silking period. A satisfactory high-clearance sprayer has been designed for applying the insecticide (Black *et al.* 1955). However, even this method is quite costly, since three to four applications are commonly recommended, although Ditman *et al.* (1955) have indicated that this can be reduced to two, by using, each time, 2 pounds of DDT and 7 quarts of mineral oil per acre.

A number of new insecticides have appeared since the advent of DDT, but relatively few seem to have been used on sweet corn in sprays containing mineral oil. The following have been tested: TDE, chlordane and toxaphene (Anderson & Hashe 1949); TDE, Perthane, endrin, isodrin and methoxychlor (Bacon *et al.* 1953); and malathion (Darsie 1953, Ditman *et al.* 1955). Of these, only TDE, Perthane and endrin approached DDT in effectiveness against the earworm.

The dusky sap beetle has become an important pest only during the past decade. Larvae, as well as adults, invade the kernels. The latter leave the ear during, or prior to, husking, but the larvae are not easily removed from the ear, even by washing. Darsie & Connell (1952) and Darsie (1953) reported on the control of this beetle in sweet corn with EPN, parathion and malathion. At least two, weekly, malathion sprays, beginning when many of the silks have browned, are required.

Experiments were continued in 1954, in a search for a material that would provide control with a single application. A heavy corn-earworm infestation interfered with evaluation of the results. However, two materials which appeared promising were tried again in 1955 tests, which are reported on herein. In this instance, the candidate materials were used in conjunction with a three-application DDT-mineral oil treatment against earworm.

MATERIALS AND METHODS.—The tests were carried out in a field of Iochief sweet corn, planted especially for this purpose at Georgetown, Delaware. Treatments were replicated three times on plots four rows wide and 50 feet long. Materials were applied to the silks with a knapsack sprayer at the times and rates indicated in table 1. Three quarts of 25% DDT emulsion and 8 quarts of mineral oil,[3] in 22 gallons of water, were used per acre for earworm control. There were three applications of this mixture. The first was made August 5, at 10% silk, but prolonged rainy periods interferred with the timing of the others; at the second, August 9, about 60% was in silk and at the last, August 15, the corn was about 2 days beyond full silk. The insecticides being evaluated, 25% aldrin emulsion and 25% Diazinon emulsion, were used at the rate of 2 and 3 quarts per acre, respectively. They were mixed and applied with the DDT-mineral oil on August 15. A standard sap-beetle treatment of malathion was included in the experiment. This was mixed with the DDT-mineral oil of August 15, and applied separately on August 19, at the rate of 1 pint of 50% emulsion per acre.

Harvest data were taken on August 25, at early dough stage, by examining 10 ears pulled at random on each plot. The amount of trim, required to remove injured portions of ears, was estimated with the scaling device shown in figure 1. This is a triangular frame constructed from $\frac{3}{4}'' \times 1\frac{3}{4}''$ shingle lath. A piece of one-fourth-inch-mesh

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[3] HBO-7, Shell Chemical Co., Inc.

Table 1.—Results of insecticide tests on Iochief sweet corn, Georgetown, Delaware, 1955.

Dosage[a] and Dates Applied	Percentage of Ears with Injury		Sap Beetle Larvae per Ear	Length Unfilled Tip (Inches)	Inches Trimmed per Ear Below Unfilled Tip			Residue[b] (p.p.m.) on	
	Earworm	Sap Beetle			From Earworm	Sap Beetle	Total	Husk	Kernels
Check	97	100	18.0	1.13	1.15	1.00	1.67		
DDT, 1.5 lb.+ HBO-7, 8 qts.—Aug. 5, 9, 15	60	97	22.0	1.38	.48	1.72	1.77		
DDT, 1.5 lb.+ HBO-7, 8 qts.—Aug. 5, 9 DDT, 1.5 lb.+ HBO-7, 8 qts.+ Malathion 0.5 lb.—Aug. 15 Malathion 0.5 lb.—Aug. 19	63	97	10.0	1.70	.51	.63	1.06		
DDT, 1.5 lb.+ HBO-7, 8 qts.—Aug. 5, 9 DDT, 1.5 lb.+ HBO-7, 8 qts.+ Aldrin 1.0 lb.—Aug. 15	53	20	.6	1.40	.60	.25	.82	0.35	<0.10
DDT, 1.5 lb.+ HBO-7, 8 qts.—Aug. 5, 9 DDT, 1.5 lb.+ HBO-7, 8 qts.+ Diazinon 1.5 lb.—Aug. 15	43	23	.4	1.54	.21	.13	.33	2.95	.06

[a] Rate per acre in water to make 25 gallons.
[b] Samples taken August 29; aldrin analysis by Shell Development Co., Denver, Colo., phenyl azide method; Diazinon analysis by Geigy Chemical Corporation, Bayonne, N. J., sulfide procedure.

hardware cloth is nailed to the back in such a manner that the horizontal strands of wire are parallel with the base. Each horizontal strand, therefore, is one-fourth of an inch farther from the base than the strand next below it. The device is calibrated, for ease in reading, by marking these distances from the base on the sloping side of the frame.

RESULTS.—The data regarding infestation and amount of injury, noted on plots receiving the various treatments, are presented in table 1.

Unfilled ear tips were found throughout the experiment. Their average length is given in the fourth column of the table. These are not characteristic of Iochief, but probably resulted from prolonged rains during the pollination period. That they were found generally, even on the unsprayed plots, indicates that they were not caused by the insecticides. The recorded average length of the unfilled tips for the unsprayed check is low, because of extensive earworm injury, which rendered it impossible to measure its full length on some ears.

Several methods of comparing the treatments are presented in the table. One of these, the trim required to remove insects and insect injury, is contained in columns five through seven. Damage to sweet corn received at processing plants is often estimated in similar terms, since this indicates how much of the ear must be discarded, and also provides a basis for estimating the amount of labor required for trimming. Because of the presence of the unfilled tips, the trim required below this is thought to provide a better basis for comparing the treatments, than would be the case with the use of total trim. The first of these three columns indicates the trim required to remove areas damaged by earworm. This includes earworm-damaged portions secondarily invaded by sap beetle. The second shows trim required to remove kernels damaged by sap beetle. This includes trim from ears damaged solely by sap beetle, as well as from those where such injury extended beyond an area of earworm feeding. The last column represents total trim, which, obviously, is not the sum of the figures in the two columns preceding.

By any of the methods of comparison, the treatments which included Diazinon and aldrin were equally effective, and the most effective, against the dusky sap beetle. For these treatments, respectively, 23 and 20% of the ears were injured by this pest, in comparison with 100% injured on the unsprayed check. There were 0.4 and 0.6 larvae per ear, respectively, as compared with 18 per ear on the check. There were, respectively, only 0.13 and 0.25 inches of trim required to remove sap beetle injured portions, in comparison with a full inch on the check.

The three applications of DDT reduced corn-earworm damage from 97% injured and 1.15 inches of trim, on the unsprayed check, to 60% injured and 0.48 inches of trim. When aldrin and Diazinon were used, the percentage of ears injured dropped to 53 and 43, respectively. With

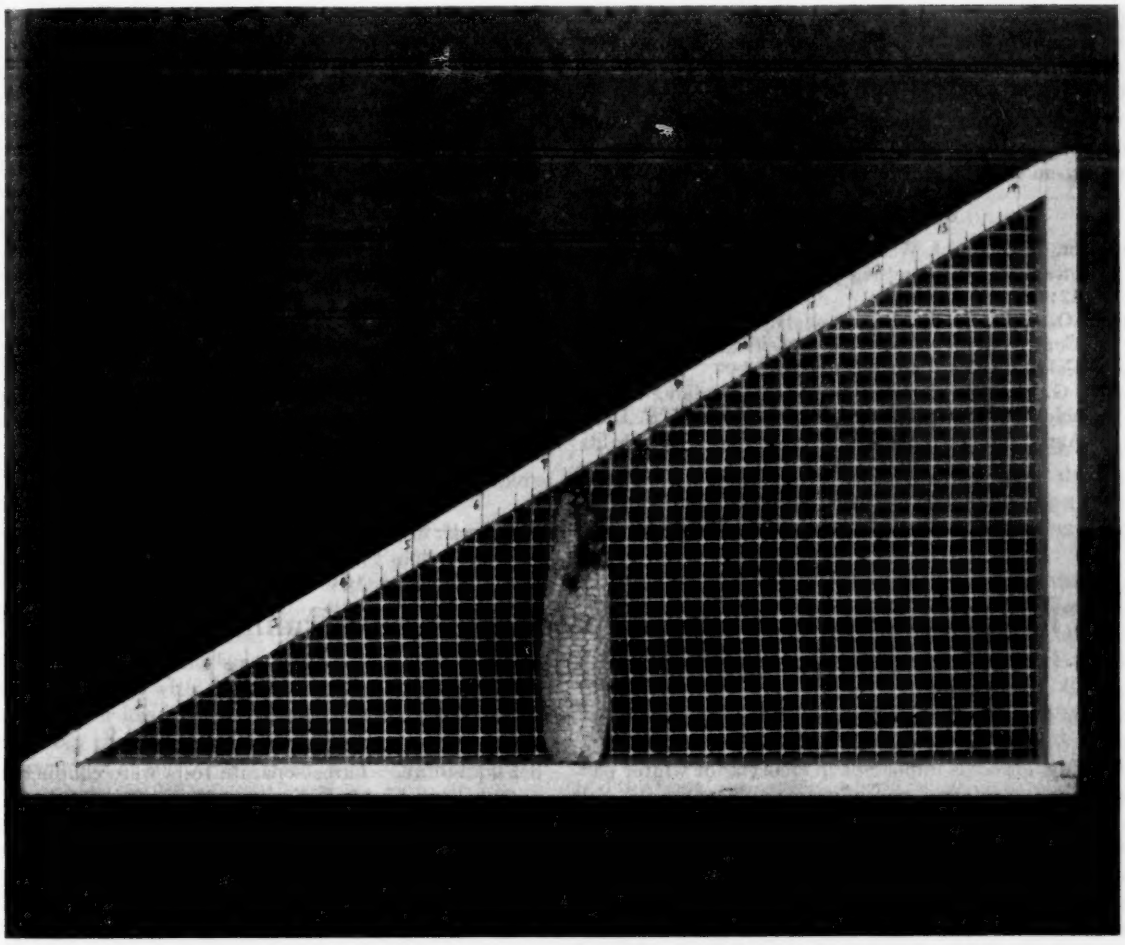

FIG. 1.—Device for measuring ear length and amount of trim.

aldrin, 0.6 inches of trim were required to remove earworm injury, while the best rating in this category was for Diazinon, where only 0.21 inches of trim were required.

Since the experiment was concerned with control of the sap beetle and time for recording data was limited, the information taken down on earworm infestation was restricted to that which appeared pertinent to the purpose of the study. Consequently, live earworms were not counted on all plots. It is believed, however, that most of the ears found injured, contained one or more living specimens, except in the case of the aldrin and Diazinon treatments. Here a count was actually made. Seventeen and 13% of the ears, respectively, from these plots, contained live earworms. Many of the ears which were recorded as damaged, from these treatments, contained dead earworms, suggesting that most of the injury that was found here occurred prior to the use of these materials.

The results indicate a good prospect for the development of a one-application control method for both corn earworm and dusky sap beetle, utilizing mineral oil to carry a suitable insecticide into the silk tube. The observed control of earworm may have resulted from the combined effects of DDT plus Diazinon or aldrin, but in the case of the sap beetle, which appears unaffected by DDT, the observed results must be attributed solely to the other materials. Much work remains, however, including the determination of the proper time and rate of application, before either Diazinon or aldrin can be recommended for use on this crop.

SUMMARY.—Small replicated plots, 0.016 acre, of Iochief sweet corn were treated for control of corn earworm, *Heliothis zea* (Boddie) three times during a 10-day period beginning at 10% silking, with a standard DDT-mineral oil spray. Certain plots were treated for control of the dusky sap beetle, *Carpophilus lugubris* Murry, by adding, with the last DDT-mineral oil application, malathion, aldrin, or Diazinon at 0.5, 1.0 and 1.5 pounds per acre, respectively. Malathion was applied again, separately, 4 days later. Where Diazinon was used, 43 and 23% respectively, of the ears were injured by earworm and sap beetle, for the removal of which 0.21 and 0.13 inches, respectively, of trim were required. Where aldrin was used, 53 and 20%, respectively, were injured by earworm and sap beetle and 0.6 and 0.25

inches, respectively, of trim were required. These figures compare with 97 and 100%, respectively, injured by earworm and sap beetle on unsprayed plots, for which 1.15 and 1.00 inches, respectively, of trim were required. The number of sap beetle larvae per ear were 0.4 where Diazinon was used and 0.6 for the aldrin treatment in comparison with 18 on the unsprayed plots.

REFERENCES CITED

Anderson, L. D., and J. W. Hashe. 1949. Control of earworm on sweet corn in southern California. Jour. Econ. Ent. **42**: 933–41.

Bacon, O. G., L. D. Anderson and H. T. Reynolds. 1953. Control of corn earworm attacking sweet corn in California. Jour. Econ. Ent. **46**: 614–20.

Barber, G. W. 1940. The use of oil or oil containing insecticides for earworm control in sweet corn. U. S. Dept. Agric. Bur. Ent. and Plant Quar. Circ. **E-525**: 1–12.

Barber, G. W. 1944. Mineral oils alone or combined with insecticides, for control of earworms in sweet corn. U. S. Dept. Agric. Tech. Bull. **880**: 1–83.

Black, D. T., L. P. Ditman and G. J. Burkhardt. 1955. A high-clearance self-propelled sprayer for sweet corn. U. S. Dept. Agric. Circ., **946**: 1–13.

Blanchard, R. A., W. A. Douglas and G. P. Wene. 1949. DDT sprays for control of the corn earworm and budworm in sweet corn. U. S. Dept. Agric. Bur. Ent. and Plant Quar. Circ. **E-780**: 1–5.

Darsie, R. F., and W. A. Connell, 1952. Preliminary tests for control of sap beetles in sweet corn. Trans. Peninsula Hort. Soc. **42**: 18–20.

Darsie, R. F. 1953. Further tests for control of sap beetles in sweet corn. Trans. Peninsula Hort. Soc. **43**: 28–32.

Ditman, L. P., H. S. Todd and F. P. Harrison. 1955. Investigations on insects attacking the sweet corn ear. Maryland Agric. Expt. Sta. Bull. **A-82**: 1–16.

The Effectiveness of Several Insecticides and Formulations in the Control of Seed Weevils on Crimson Clover[1]

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Crimson clover is important in Georgia for winter pasture, soil conservation and improvement, and as a cash seed crop. During the past several years two species of insects, a clover weevil, *Hypera meles* (F.),[2] and the lesser clover leaf weevil, *Hypera nigrirostris* (F.), have been present in large numbers in crimson clover fields in Spalding and Fayette Counties. Although a complete survey has not been made, the two species are considered to be present in many other north Georgia counties. Apparently the weevils are more abundant in fields where crimson clover has been established and reseeding for several years. Counts during 1953 showed larvae present on clover heads at the rate of 1,054,720 *Hypera meles* and 955,840 *H. nigrirostris* per acre. Both larvae and adults occur on the clover heads during the period of bloom and seed development. Little work has been done on these insects in the southern states.

Johnson & Nettles (1953) reported the two species were discovered in South Carolina on crimson clover in 1951 as the seed crop was being harvested. They considered the larvae more injurious than the adults to the florets, buds, and newly forming seed. In field tests, chlordane showed the most promise from the standpoint of control and yields. Detwiler (1923) published on investigations in New York and gave the description and life history of the two species. More recently in New York, Schwardt, *et al.* (1947) in field tests on red clover obtained considerable reduction in infestation of *Hypera meles* with applications of DDT and BHC. In view of the need for more information on these weevils, investigations have been in progress at the Georgia Experiment Station to obtain data on life history, measurement of damage, and control. This paper is a report of progress in control with various insecticides and formulations.

PROCEDURE.—Three separate tests were conducted in a 60-acre field of Dixie reseeding crimson clover in Fayette County, Georgia. The tests involved dust, emulsifiable concentrate spray, and granulated formulations of different insecticides. Plots were the same size in each experiment, 10 feet wide by 20 feet long. There were four replicates of each treatment, and the plots were randomized and laid out in a block design. An alley 5 feet wide separated each block. In addition, the dust test had buffer areas the same size as the treated areas between plots to minimize the problem of drift.

Table 1 shows the insecticide dust formulations used and the amount of actual insecticide applied per acre. Although the percentages of the different dusts varied, all were applied at the rate of 30 pounds of the commercially prepared material per acre. Small hand dust guns were used to apply the dusts on April 27. The application was made when the clover was past peak bloom at a time when approximately 50% of the florets were pollinated.

In the spray test, emulsifiable concentrates were mixed with water and applied with knapsack sprayers at the rate of about 55 gallons of spray per acre. Insecticides in the spray test were applied on April 27. Table 2 gives the amount of actual material applied per acre.

Granulated formulations were mixed with 4-12-12 fertilizer and applied with a small fertilizer distributor on April 15. Table 3 shows the amount of actual insecticide applied with the fertilizer. The fertilizer was ap-

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[2] This species was popularly referred to as the clover head weevil by Detwiler (1923).

Table 1.—Control of a mixed population of *Hypera meles* and *H. nigrirostris* on crimson clover with various insecticide dusts. Fayette County, Georgia. 1955.

Treatment	Pounds Actual per Acre	Live Larvae per 100 Heads	
		meles	*nigrirostris*
Heptachlor, 2.5%	0.75	1.0	1.2
Dieldrin, 1.5%	0.45	2.0	1.5
Malathion, 4%	1.2	2.5	3.0
Chlordane, 5%	1.5	6.0	3.2
Aldrin, 2.5%	0.75	6.8	3.5
Parathion, 1%	0.3	16.8	2.2
Toxaphene, 20%	6.0	22.0	3.8
Check		53.8	19.8
L.S.D. at 5% level		12.5	4.8

plied at the rate of 800 pounds per acre. The check plots received only the fertilizer application.

In order to evaluate the effectiveness of the insecticides, samples of 100 clover heads per plot or 400 per treatment were collected in the field, placed in paper bags, and examined in the laboratory for presence of live larvae of the two species. Clover heads were collected and examined from the dust and spray tests on May 5 and from the test with granulated insecticides on May 9. Mature clover heads were harvested on May 17 for seed yields from the three experiments. A total of 100 heads from each plot or 400 per treatment were collected, placed in paper bags, and kept in the laboratory until dry on June 6 when they were threshed with a small electric thresher. The seeds were then examined for damage, counted, and weighed.

RESULTS AND DISCUSSION.—During the course of these experiments, there was a somewhat higher population of *Hypera meles* than of *H. nigrirostris*. Although the two species occur together on the clover heads, *H. nigrirostris* is present slightly earlier. It is apparent from the counts of larvae that *H. meles* was at its peak while *H. nigrirostris* was beginning to decline in numbers. Sweeping records in the field showed *H. meles* larvae increased in numbers about 10 times from April 25 to May 2 and *H. nigrirostris* larvae decreased during this period.

Table 1 shows the average number of larvae present after the one application of the different dust formulations. While there is some difference in degree of control, all materials significantly reduced the infestation of both weevils. The emulsifiable concentrate sprays also gave significant control of both species. It is not known whether the results from Am. Cyanamid 3911 were from systemic or contact action.

Table 2.—Control of a mixed population of *Hypera meles* and *H. nigrirostris* on crimson clover with various insecticide sprays. Fayette County, Georgia, 1955.

Treatment	Pounds Actual per Acre	Live Larvae per 100 Heads	
		meles	*nigrirostris*
Am. Cyanamid 3911	1.0	0.2	0.5
Dieldrin	0.3	1.5	1.0
Endrin	0.4	2.2	0.8
Malathion	1.25	4.2	3.0
Methoxychlor	1.0	4.2	1.0
Chlorthion	0.5	7.8	4.5
Toxaphene	2.0	12.5	6.5
Check		34.8	12.5
L.S.D. at 5% level		8.4	3.0

Table 3.—Control of a mixed population of *Hypera meles* and *H. nigrirostris* on crimson clover with granular insecticide-fertilizer mixtures. Fayette County, Georgia. 1955.

Treatment	Pounds Actual per Acre	Live Larvae per 100 Heads	
		meles	*nigrirostris*
Heptachlor, 2.5%	2.5	0.0	0.0
Dieldrin, 5%	2.5	1.0	0.0
Aldrin, 2%	2.5	2.2	0.2
Toxaphene, 20%	10.0	37.5	1.0
Check		52.8	2.2
L.S.D. at 5% level		17.8	1.1

Granulated formulations of heptachlor, dieldrin, and aldrin all gave outstanding control of *H. meles*. Toxaphene did not reduce this species significantly. All materials were effective against *H. nigrirostris*, although the infestation was light. Apparently the control in this test was against the adults; this prevented egg laying and a subsequent build-up of larvae on the heads. Approximately 3 weeks elapsed between the time of application and the larval count. This would give the adults sufficient time to come in contact with the granulated insecticides on the soil surface.

Although good control of the two species was obtained with the different insecticide formulations, more information is needed on time and number of applications. Applications made earlier, when the clover starts to bloom, may result in better control. Granulated materials should be applied about the third week in March as soon as the cattle are removed from the pasture and the clover is allowed to bloom and produce a seed crop. The fertilizer was used as a medium for better coverage or distribution of the granulated insecticides. Ordinarily, fertilizer would be applied to crimson clover in the early fall instead of the spring. Some increase in yield of sound seed was obtained in the different tests with all materials except granulated toxaphene. However, according to the F test the increased yields were not significant.

SUMMARY.—Field tests for control of a mixed population of *Hypera meles* (F.) and *H. nigrirostris* (F.) on crimson clover were conducted using one application of different insecticide dust, spray, and granulated formulations. Dusts of heptachlor, dieldrin, malathion, chlordane, aldrin, parathion, and toxaphene gave a significant reduction of larvae of both species on the clover heads. Sprays of Am. Cyanamid 3911, dieldrin, endrin, malathion, methoxychlor, Chlorthion, and toxaphene also reduced the species significantly. Granulated insecticide-fertilizer mixtures of heptachlor, dieldrin, and aldrin were effective against both weevils. Granulated toxaphene did not give satisfactory control. Increased seed yields were obtained in the three experiments with all materials except granulated toxaphene, although they were not significant.

REFERENCES CITED

Detwiler, John D. 1923. Three Little-Known Clover Insects. Cornell Univ. Agric. Expt. Sta. Bull. **420.**

Johnson, W. C., and W. C. Nettles. 1953. Demonstrations in Control of Clover Weevils, 1952 Results. Clemson Agric. College Ext. Serv. Misc. Publ. (Unnumbered)

Schwardt, H. H., L. D. Newsom, and L. B. Norton. 1947. Increasing Red Clover Yields by Treatment With DDT or Hexachlorocyclohexane. Jour. Econ. Ent. **40**(3): 363–5.

The Relative Toxicity and Mode of Action of Some Chlorinated Phosphates[1]

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Five closely related chlorinated phosphates, synthesized by Barthel *et al.* (1955), have been examined in this laboratory for their physiological and toxic action because of their relationship to the insecticide DDVP. Because the mode of action of the organic phosphorus insecticides has been assumed to be that of cholinesterase inhibition (Mazur & Bodansky 1946, Chadwick & Hill 1947, Metcalf & March 1949), these chlorinated phosphates were examined for their ability to inhibit cholinesterase and to seek possible correlation between such ability and their relative toxicity to the house fly, *Musca domestica* L.

MATERIALS.—All the compounds were esters of 1,2,2,2-tetrachloroethyl phosphoric acid of the general formula

$$(R{-}O)_2{=}P{-}OCHClCCl_3$$

R being a methyl, ethyl, propyl, isopropyl, or butyl group.

The first three materials were of a high degree of purity, the fourth was a crude product, and the fifth had been redistilled. They were prepared by chlorinating the dialkyl chlorovinyl phosphates (Barthel *et al.* 1951) by the method of Perkow (1954). The physical constants of the esters are found in table 1.

Two strains of house flies were used for the toxicity tests, an insecticide-susceptible and a resistant strain. The susceptible flies were of the 1948 NAIDM strain, and the resistant flies were of the Orlando-Beltsville strain that had been selected for DDT resistance for more than 150 generations.

Table 1.—Physical constants of the esters of 1,2,2,2-tetrachloroethyl phosphoric acid.

Ester	Boiling Point		Melting Point	
	° C.	Mm. Hg	(° C.)	N_D^{25}
Dimethyl	99–102	0.2	42–43	1.4701
Diethyl	116–119	0.4	—	1.431
Dipropyl	127–135	1	—	1.4626
Diisopropyl	Not distilled		—	1.4628
Dibutyl	151–158	1.2	—	1.4673

PROCEDURE.—In an earlier paper (Babers & Mitlin 1955) we reported the apparent lack of cholinesterase activity in fly brain and the necessity of using American cockroach brain as an enzyme source. This apparent lack has now extended to cockroach brain and ventral nerve cords and to honey bee brain. It has since been determined that the apparent lack of enzyme activity in the insects is an artifact peculiar to our laboratory. L. E. Chadwick, of the Army Chemical Center, tested our strain of flies in his laboratory and found that they contained the normal potent cholinesterase.

In order to continue our tests we used washed bovine red blood cells, diluted 25 times with 0.001 *M* sodium acetate, as a source of enzyme. A mixture of 2.6 ml. of the red-cell preparation, 0.1 ml. of acetone solution of the chlorinated phosphate and 0.3 ml. of 0.004 *M* acetylcholine bromide solution was incubated at 37.5° C., and enzyme activity was determined by Hestrin's method (1947). The molar concentration required to give 50% inhibition in 30 minutes was determined. For the toxicity tests the compounds were dissolved in acetone to form solutions containing the proper dosages as determined by preliminary tests.

The insects, under carbon dioxide anesthesia, were treated with 1.227 microliters of the acetone solution by the device described by Roan & Maeda (1953). Forty female house flies averaging 3 to 4 days of age were used for each dosage. The tests were made in three series against different populations of flies. Mortality data were taken after 24 hours.

EVALUATION OF TOXICITY.—The mortality results are given in table 2. To evaluate relative toxicity and determine the precision of the estimates, the mortality data were subjected to probit analysis as described by Finney (1952).

The regression lines were fitted graphically to the data in table 2, and provisional lines determined. The individual weighted regression lines were computed. The data were found to be homogeneous for all but one compound, the diethyl ester in series 1, wherein a heterogeneity factor of 3.73. was used

The equations showing the regression of mortality,

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[2] Grateful acknowledgment is made to Anne M. Baroody for her assistance.

Table 2.—Mortality of house flies 24 hours after treatment with chlorinated phosphates.

Ester	Susceptible Flies: μg. per Fly	Susceptible Flies: Per Cent Mortality in 1 Day	Resistant Flies: μg. per Fly	Resistant Flies: Per Cent Mortality in 1 Day
		Series 1		
Dimethyl	0.250	95.0	0.335	77.5
	.200	91.0	.268	65.0
	.160	77.5	.214	57.5
	.128	65.0	.171	30.0
	.102	20.0		
Diethyl	.250	97.5	.524	100.0
	.200	90.0	.419	87.5
	.160	25.0	.335	70.0
	.128	15.0	.268	47.5
	.102	5.0		
Dipropyl	.503	92.5	2.00	75.0
	.402	65.0	1.60	65.0
	.362	56.5	1.280	60.0
	.290	30.0	1.024	17.5
	.236	10.0		
		Series 2		
Dimethyl	0.200	90.0	0.335	70.0
	.160	62.5	.268	57.5
	.128	40.0	.214	46.5
	.102	12.5	.171	32.5
Diisopropyl	1.0	75.0	.655	97.5
	.75	47.5	.424	67.5
	.60	15.0	.339	55.0
	.45	12.5	.254	42.5
Dibutyl			1.92	90.0
			1.53	67.5
			1.224	42.5
			.979	30.0
		Series 3		
Dimethyl	0.200	82.5		
	.160	35.0		
	.128	40.0		
	.102	15.0		
Dibutyl	1.20	100		
	.960	97.5		
	.770	92.5		
	.616	50.0		

expressed in probits, on dosage, in micrograms per 10 flies, expressed in logarithms are as follows:

Ester	*Susceptible flies*	*Resistant flies*
Series 1		
Dimethyl	$Y = 6.277X + 4.442$	$Y = 5.191X + 3.253$
Diethyl	$Y = 10.183X + 2.801$	$Y = 5.191X + 2.873$
Dipropyl	$Y = 8.012X + 0.709$	$Y = 5.191X - 0.875$
Series 2		
Dimethyl	$Y = 6.660X + 4.017$	$Y = 4.558X + 3.324$
Diisopropyl	$Y = 6.660X - 0.918$	$Y = 4.558X + 2.863$
Dibutyl		$Y = 4.558X + 0.048$
Series 3		
Dimethyl	$Y = 5.911X + 3.887$	
Dibutyl	$Y = 10.199X - 2.908$	

From these equations LD 50's were estimated and relative toxicities calculated as inverse ratios of the LD 50's, as given in table 3.

COMPARISON OF CHOLINESTERASE INHIBITION.—Median inhibition dosages (IN 50's) were determined graphically on log-probability paper and are reported in table 4. These data were not analyzed statistically. No extensive data were compiled because of our inability to use house fly cholinesterase. However, rather large statistical errors were apparent.

Table 3.—Relative toxicity of the chlorinated phosphates to house flies.

Ester	Susceptible Flies: LD-50 (μg. per fly)	Susceptible Flies: Ratio of Toxicity	Resistant Flies: LD-50 (μg. per fly)	Resistant Flies: Ratio of Toxicity
		Series 1		
Dimethyl	0.123±0.005	1	0.217±0.010	1
Diethyl	.164± .009	0.75 ±0.041	.257± .016	0.844±0.066
Dipropyl	.343± .010	.359± .034	1.36 ± .027	.160± .010
		Series 2		
Dimethyl	0.140±0.006	1	0.233±0.012	1
Diisopropyl	.774± .032	0.181±0.012	.294± .056	0.793±0.154
Dibutyl			1.22 ± .068	.191± .014
		Series 3		
Dimethyl	0.154±0.016	1		
Dibutyl	.596± .060	0.261±0.038		

RELATIONSHIP OF CHEMICAL STRUCTURE TO TOXICITY AND CHOLINESTERASE ACITIVITY.—This study shows a correlation between toxicity to house flies and chemical structure. The toxicity to susceptible flies generally decreased with an increase in the length of the carbon chain of the alkyl group. The diethyl, dipropyl, and dibutyl compounds were, respectively, 0.75, 0.36, and 0.26 as toxic as the dimethyl compound. The diisopropyl compound was the least toxic of the group, either owing to steric hindrance of rotation of the two isopropyl groups or simply because it was a crude product.

The picture is not so clear concerning cholinesterase inhibition. The inability to obtain fly cholinesterase makes it difficult to draw conclusions. Metcalf & March (1950) and Babers & Pratt (1951) have shown that there are distinct biochemical differences between specific cholinesterases from unlike animal species. These differences are reflected in the relative cholinesterase inhibition of organic phosphorus compounds.

Because of the apparent large errors for the curves, there appears to be little difference among the four straight-carbon-chain esters, and no correlation can be made between cholinesterase inhibition and chemical

Table 4.—Inhibition of cholinesterase by the chlorinated phosphates.

Ester	IN 50 (Moles)	Ratio of Inhibition
Methyl	8.24×10^{-7}	1
Ethyl	6.93×10^{-8}	11.89
Propyl	2.25×10^{-7}	3.67
Isopropyl	7.0×10^{-2}	1.24×10^{-5}
Butyl	9.5×10^{-6}	8.7×10^{-2}

structure. Therefore, although inhibition of cholinesterase may be a factor in the mode of action of the chlorinated phosphates, no direct relation was demonstrated between the inhibition of the enzyme in red blood cells and length of the alkyl carbon chain in the compounds tested.

COMPARISON OF SUSCEPTIBLE AND RESISTANT FLIES.—In general, the resistant flies showed but little more tolerance to the esters than the susceptible flies. Resistance ranged from approximately 4 times for the dipropyl ester to 1.6 times for the diethyl ester. A curious reversal appeared for the diisopropyl ester, wherein this material was 2.6 times as toxic to resistant as to susceptible flies.

VALUE AS PRACTICAL INSECTICIDES[3].—Although preliminary laboratory investigations cannot determine the potential efficacy of insecticidal chemicals under practical conditions, it is interesting to note that the LD 50's of the dimethyl, diethyl, and dipropyl esters for house flies were equal to or lower than the LD 50 of Bayer L 13/59 found by the same method (Babers & Mitlin 1955). Comparisons are of necessity rough, since the tests were made on different populations of flies. However, in the earlier study the LD 50 of technical L 13/59 was 0.395 mg. per susceptible fly, whereas the average LD 50 for the dimethyl compound in this study was 0.139 μg. per fly, a nearly three-fold difference.

SUMMARY.—Five esters of 1,2,2,2-tetrachloroethyl phosphoric acid were tested for cholinesterase inhibition and relative toxicity to the house fly, *Musca domestica* L. The diethyl, dipropyl, diisopropyl, and dibutyl esters were 0.75, 0.36, 0.18, and 0.26 as toxic as the methyl ester when tested against an insecticide-susceptible strain, and 0.84, 0.16, 0.79, and 0.19 as toxic to a resistant strain. Generally, toxicity decreased with the increase in the length of the carbon chain of the alkyl group.

All the materials were inhibitors of bovine red-blood-cell cholinesterase. No definite relationship between chemical structure and cholinesterase inhibiton was shown.

Flies of the resistant strain generally were only slightly more resistant to these esters than flies of the susceptible strain.

REFERENCES CITED

Babers, F. H., and Norman Mitlin. 1955. The physiology and toxicology of certain derivatives of Bayer L 13/59. Jour. Econ. Ent. **48**: 430–1.

Babers, F. H., and John J. Pratt. 1951. Comparison of the cholinesterase in the heads of the house fly, the cockroach, and the honey bee. Physiol. Zool. **24**: 127–31.

Barthel, W. A., Ben Alexander, Paul Giang, and S. A. Hall. 1955. Insecticidal phosphates obtained by a new rearrangement reaction. Jour. Amer. Chem. Soc. **77**: 2424–7.

Chadwick, L. E., and D. L. Hill. 1947. Inhibition of cholinesterase by diisopropyl fluorophosphate, physostigmine, and hexaethyl tetraphosphate in the roach. Jour. Neurophysiol. **10**: 325–46.

Finney, D. J. 1952. Probit analysis. 318 pp. Cambridge.

Hestrin, Shlomo. 1949. The reaction of acetylcholine and other carboxylic acid derivatives with hydroxylamine and its analytical application. Jour. Biol. Chem. **180**: 249–61.

Mazur, A., and O. Bodansky. 1946. The mechanism of *in vitro* and *in vivo* inhibition of cholinesterase activity by diisopropyl fluorophosphate. Jour. Biol. Chem. **163**: 261–76.

Metcalf, R. L., and Ralph B. March. 1949. Studies of the mode of action of parathion and its derivatives and their toxicity to insects. Jour. Econ. Ent. **42**: 721–8.

Metcalf, R. L., and Ralph B. March. 1950. Properties of acetylcholine esterases from the bee, the fly and the mouse and their relation to insecticide action. Jour. Econ. Ent. **43**: 670–6.

Perkow, W. 1954. Umsetzungen mit Alkylphosphiten. I. Mitteil.; Umlagerungen bei der Reaktion mit Chloral und Bromal. Deutsche Chem. Gesell. Ber. **87**: 755.

Roan, C. C., and Shizuko Maeda. 1953. A microdevice for rapid application of toxicants to individual insects. U. S. Bur. Ent. and Plant Quar. **E-306.**

[3] It has been found that these compounds are highly toxic to warm-blooded animals.

Screening Wheat Varieties and Hybrids for Resistance to the Greenbug[1]

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The primary problem in any study of insect resistance in crop plants is the finding of plant varieties that are sources of resistance. The possibility of finding such sources is more or less proportional to the number and diversity of plant materials that can be studied. These conditions apply to the original search and also to the search for desirable recombinations after hybridization between plant sources of resistance and standard commercial varieties.

A screening method must provide for (1) a study of many strains in a minimum of time and space, (2) a reasonably accurate classification of levels of resistance with reproducibility of results and (3) an opportunity to discern the presence of various components of resistance: preference, tolerance, and antibiosis (Painter 1951).

Several methods of screening small-grains for resistance to the greenbug *Toxoptera graminum* (Rond.), have been reported by Dahms *et al.* (1955). The methods they reported evaluated separately resistance as measured by preference, fecundity of aphids reared, and several measures of tolerance. Tolerance as measured by Dahms *et al.* and as reported below is influenced both by preference and antibiosis.

MATERIAL AND METHODS.—Greenbugs for tests originated from a single female and, as far as known, repro-

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[2] Professor and Graduate Research Assistant, respectively. The writers wish to acknowledge the assistance of E. G. Heyne, Department of Agronomy, Kansas State College, in the agronomic phase of this study.

duction was entirely parthenogenetic. All tests were conducted in a basement insectary with fluorescent lights used 16 hours per day and with heat, when needed, to maintain a temperature of 70 to 80° F. Greenbugs were cultured on flats of Reno barley planted at 10-day intervals. The flats were placed adjacently so that the aphids could move over to the new plants soon after plants emerged or when the population killed the plants in the flat where they had been feeding. This population constituted the source of aphids for infesting plant material to be tested.

In testing wheat varieties, the usual method was to plant 20 half-rows per flat, with 10 to 14 plants per row. These included 18 strains to be tested with Pawnee as a susceptible check and Dickinson selection as a resistant check. The latter is a resistant spring wheat strain derived from the Dickinson No. 485, C. I. 3707, mentioned by Dahms *et al.* (*op. cit.*). A gap of about 2 inches through the center of the flat was left between the ends of the two series of rows.

The seedlings were infested on the sixth or seventh day after planting when they were 2 to 4 inches tall, by putting leaves carrying many aphids in a long narrow wire basket placed in the center of the flat. Since there was a tendency for the outside rows of the flat to become more heavily infested, the infested leaves should not reach the ends of the basket. Infestation spread outward from the central ends of the rows of plants.

The time from infestation until tolerance records were taken depended upon the number of aphids present, the temperature, and size of plants being tested. It was usually about 14 days. Records were taken at the time when there appeared to be a maximum difference between the resistant and susceptible checks. The plants in the rows were graded into five grades or degrees of injury as indicated in table 1 which also gives the meaning of the grades. Additional notes could be made of the types of injury, any exceptional concentration or lack of aphids or other differences among varieties.

In screening segregating F_2 populations the method was altered to plant 10 full rows in each flat. The outer rows were planted with Pawnee wheat. The central two rows were planted with the susceptible and resistant parents. Infestation was made by scattering about 500 green bugs in the center of the flat, leaving a margin of about 2 inches around the outside free of the insects. The greenbugs were allowed to reproduce until they killed most of the plants in the border rows of Pawnee and the susceptible parents. The aphids were then killed with nicotine sulfate and the remaining F_2 and resistant parental plants grown to maturity in the greenhouse.

EXPERIMENTAL RESULTS.—A number of strains of wheat for testing as described above were secured from various sources, especially from David J. Ward, Germplasm Maintenance and Evaluation, U. S. Department of Agriculture. The results of a series of tests, using the procedures outlined, were as recorded in table 1. The ratings of the resistant and susceptible checks differed by at least one grade, but usually by two grades. Most of the varieties tested appeared to be more susceptible than Pawnee with only about 4% of the entries within range of the resistant check. They and a few others that were not uniform in reaction to greenbugs are being retested more thoroughly after the original screening experiments were concluded.

Hybrids between the greenbug-resistant Dickinson selection and three winter wheats adapted to Kansas were made by members of the Agronomy Department in 1954. The F_1 plants were grown in the greenhouse in 1955. The crosses involved are listed in table 2.

Table 1.—Tolerance to the greenbug of 2,141[a] foreign wheat introductions and checks tested 1954 and 1955.

Grade No.	Dickinson Checks	Pawnee Checks	Wheat Varieties		Description of Grade
			Winter	Spring	
	No. Rows	No. Rows	Number	Number	
1	6	0	0	0	Aphid present, but no chlorosis
2	24	0	3	0	Slight chlorosis no stunting
3	16	4	48	29	Some chlorosis or stunting or both
4	3	35	425	333	Considerably stunted or chlorotic
5	0	20	642	661	As 4, but one or more dead plants
Total	49	59	1,118	1,023	
Average Grade	2.33	4.27	4.53	4.62	

[a] A list of these introductions may be obtained from the Department of Entomology, Kansas State College, Manhattan.

Table 2.—Reaction of F_2 plants following greenbug infestation, Manhattan, Kansas 1954–1955.

Hybrid	Plants			
	Total	Alive	Dead	Chi Square
Pawnee C.I. 11669×Dickinson sel.	163	39	124	0.100[a]
Chiefkan-Oro-Tenmarq, C.I. 12518 ×Dickinson sel.	188	38	150	2.706[a]
Concho C.I. 12517×Dickinson sel.	521	130	391	0.00064[a]
Totals	872	207	665	

[a] Non-significant.

Reactions of the F_1 plants and parents were tested by caging five winged greenbugs on branches of each plant and recording the progeny at the end of a week. The cages were made of dializing tubing and nylon cloth. About a third fewer nymphs were produced on the F_1 hybrids and on Dickinson than on the susceptible winter parents. The greenbugs did not produce chlorosis in the feeding areas on Dickinson and produced much less chlorosis on the F_1 than on the winter wheat parents. In two respects, aphid fecundity and production of chlorosis, the F_1 plants showed a reaction more like the resistant than the susceptible parents.

The F_2 progenies from the hybrids discussed above were screened during the period September–December 1955 as described above, to discard the susceptible segregates. Following the treatment with aphids, only one plant survived from over 500 plants of Pawnee and other susceptible parental checks present in the flats. Most of the Dickinson parental plants survived. The F_2 plants surviving were grown to maturity in the greenhouse.

Among 872 F_2 plants exposed to the greenbug, 207 plants survived the infestation. About 700 additional F_2 plants of the Pawnee×Dickinson cross were screened but

the numbers of plants were not recorded in detail. The results, however, are known to be similar. The near approach to a 3 to 1 ratio in F_2 suggests a single major genetic factor difference for this type of reaction to the greenbug in wheat. The difference in reaction of the F_1 plants and the majority of F_2 plants, even though the criteria of resistance were different, might indicate a more complex genetic situation. Final analysis will require a study of the F_3 and subsequent generations.

SUMMARY.—Methods are described for screening numbers of wheat strains for resistance to the greenbug, *Toxoptera graminum*. Among more than 2,000 tested, most of the strains were more susceptible than Pawnee, a wheat commonly grown in Kansas. About 4% of the strains appeared in this first test to carry some resistance. Methods are also described for screening the F_2 population of hybrids between wheats susceptible and resistant to the greenbug so that only the more resistant segregates were grown to maturity. In a study of three such crosses, the proportion of plants surviving suggested a single factor difference for the type of reaction to greenbugs studied. Further studies are needed to confirm this result.

REFERENCES CITED

Dahms, R. G., T. H. Johnston, A. M. Schlehuber, and E. A. Woods, Jr. 1955. Reaction of small-grain varieties and hybrids to greenbug attack. Oklahoma Agric. Expt. Sta. Bull. 55.

Painter, Reginald H. 1951. Insect resistance in crop plants. Macmillan Co., New York, N. Y. 520 pp.

Pentachlorocyclohexene, an Intermediate in the Metabolism of Lindane by House Flies[1]

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The chlorinated hydrocarbon insecticides can be divided into two groups on the basis of house fly tolerance. One group consists of DDT and related compounds such as methoxychlor and TDE, while the other group comprises lindane, heptachlor, aldrin, dieldrin and chlordane. When resistance to any member of one group is developed, some degree of resistance to other members of that group also appears, but the flies remain more or less susceptible to the compounds of the other group as pointed out by Busvine (1954). Flies already selected for tolerance to DDT may be selected for tolerance to the lindane group of compounds in fewer generations than is required with susceptible flies (Decker & Bruce 1952). It would seem, therefore, that there must be some similarity in the mechanism responsible for resistance to these compounds.

The degree of tolerance of house flies to DDT has been found to parallel the concentration of the enzyme DDT-dehydrochlorinase in the tolerant flies (Sternburg *et al.* 1954). The exact mechanism by which the enzyme catalyzes the elimination of HCl from DDT to form the non-toxic compound DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene] is not known. The ease by which the enzyme dehydrochlorinates DDT and various analogues does not correlate with the rates found for dehydrochlorination by alkali (Sternburg *et al.* 1954). This suggests that the basic mechanisms are different, yet the end product is the same. With another substrate, enzymatic attack could conceivably alter the compound by means other than dehydrochlorination. The foremention findings would suggest that the metabolic fate of lindane might be involved with similar enzyme attack resulting in the elimination of HCl from the molecule. The enzyme DDT-dehydrochlorinase, isolated from DDT-tolerant house flies, does not, however, attack lindane.

Oppenoorth (1954, 1955) found that lindane and the alpha and delta isomers of hexachlorocyclohexane are metabolized at a rapid rate by tolerant flies and to a lesser extent by susceptible flies. The nature of the metabolites was, however, not determined. Bradbury & Standen (1955) apparently anticipating trichlorobenzene as a metabolic product of lindane metabolism were unable to find evidence for its presence in tolerant house flies. If the mechanism of detoxication of lindane involves the elimination of only one molecule of HCl to produce pentachlorocyclohexene, then it is possible that part of the product reported as lindane in flies, both by Oppenoorth, and Bradbury & Standen was actually a metabolite. By the use of spectrophotometric and chromatographic techniques, this paper presents evidence that pentachlorocyclohexene is an intermediate in the metabolism of lindane.

METHODS.—To determine the rate of penetration and metabolic fate of lindane in resistant and susceptible house flies, ethanol solutions of varying concentrations of lindane were applied so that 0.4 μl. of solution per fly gave the desired dosage. Several dosage levels were used ranging from 0.1 to 4.0 μg. of lindane per fly. Groups of 25 to 200 flies were treated topically, the solution being applied to the mesonotum with a calibrated tuberculin syringe driven by a micrometer while the flies were under light CO_2 anesthesia. The flies were held in cheesecloth-stoppered flasks for the desired period of time. Flies retained for longer than 8 hours before being sacrificed were given water in a vial plugged with cotton. For residual treatment screened rearing cages were coated with a deposit of lindane sufficient to give a white crystalline deposit on all surfaces. Newly emerged resistant adults were placed in these cages, and provided with fresh milk each day. At the end of 2 to 5 days, the survivors were removed to untreated cages and allowed to remain for

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24 hours, after which time the flies were extracted and the extracts analyzed for lindane and pentachlorocyclohexene.

The method developed by Schechter & Hornstein (1952) which was used with success by Oppenoorth (1954, 1955) in similar studies on the metabolic fate of lindane in house flies was employed in this study. This method involves the dechlorination of lindane to benzene, nitration of the benzene to dinitrobenzene, and reaction of the dinitrobenzene with methyl ethyl ketone in the presence of potassium hydroxide to form a colored complex. As little as 5 μg. of lindane can be determined by this method.

Pentachlorocyclohexene[2] when dechlorinated by zinc yields chlorobenzene, which upon nitration is converted to dinitrochlorobenzene. This compound will also give a colored complex with methyl ethyl ketone in the presence of potassium hydroxide. Absorption curves of the complexes obtained from lindane and pentachlorocyclohexene are shown in fig. 1.

Lindane treated in accordance with Schechter-Hornstein methods has two absorption maxima, one at 360 mμ (molecular extinction 8650) and another at 568 mμ (mol. ext. 17,900), and has almost no absorbance from 400 to 450 mμ. On the other hand pentachlorocyclohexene has three absorption maxima, one at 365 mμ (mol. ext. 7890), a smaller peak at 420 mμ (mol. ext. 4840), and another at 550 mμ (mol. ext. 8060). Because the absorbance of pentachlorocyclohexene at 360 mμ and 560 mμ is almost equal, whereas the absorbance of lindane at 360 mμ is less than half that of lindane at 560 mμ, quantitative measurements of the amounts of each compound present in mixtures are possible. The absorbance of the mixture at 360 and 560 mμ is measured, and by solving simultaneous equations based on the absorbance of known amounts of each compound at these wavelengths, the amounts present in the unknown are calculated. It should be pointed out that when lindane is degraded to pentachlorocyclohexene, the latter will be determined as lindane equivalent by the usual means of applying the Schechter-Hornstein method; that is, by taking one reading at 565 mμ and then determining the amount present by reference to a calibration curve made from lindane.

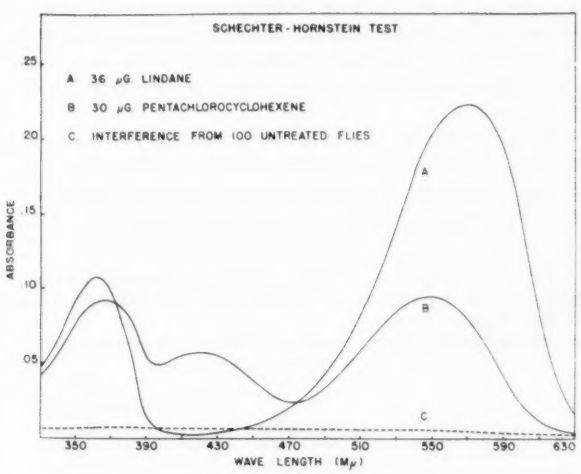

FIG. 1.—Absorption curves of Schechter-Hornstein chromagens. (A) from 36 μg. lindane; (B) from 30 μg. pentachlorocyclohexene; (C) from 100 untreated resistant house flies.

Attempts to separate lindane from pentachlorocyclohexene by the chromatographic methods of Mitchell (1952) and Bradbury & Standen (1955) were unsuccessful. However, it was found that the nitrated products of these compounds after dechlorination as in the Schechter-Hornstein test could be easily separated and distinguished by using an ascending paper chromatographic technique.

These two products, namely, dinitrobenzene and dinitrochlorobenzene, were separated by paper chromatography, using 3:1 H_2O: methanol as the mobile solvent. After spotting the material to be chromatographed on a strip of Whatman No. 1 paper (3×30 centimeters), it was sprayed with a 5% solution of cottonseed oil (Wesson Oil) in ethyl ether at the rate of 5 ml. per 300 cm^2. The paper was then placed in a stoppered glass tube at 25° C. and the solvent allowed to ascend approximately 20 cm. Stopcock grease was found to cause serious streaking of chromatograms and it was necessary to use water as a stopcock lubricant during the extraction from the nitration acid.

After development of the chromatogram, the solvent was allowed to evaporate from the paper at room temperature. The presence of the compounds on the paper could then be determined by spraying the paper with any one of three reagents, the first being the most sensitive, as follows:

1. A 1% solution of sodium polysulfide in water gave a purple color with dinitrobenzene and an orange color with dinitrochlorobenzene.

2. A 10% solution of sodium methylate in methanol gave a yellow color with dinitrobenzene, and an orange color with dinitrochlorobenzene.

3. Methyl ethyl ketone followed at once by 1% potassium hydroxide in methanol gave a purple color with dinitrobenzene and a red color with dinitrochlorobenzene. However, the colors fade rapidly and thus limit the use of this method.

Under these conditions the R_f for dinitrobenzene was 0.13 (0.09–0.17) and for dinitrochlorobenzene 0.33 (0.26–0.40) in 15 trials. The method is sufficiently sensitive to detect and separate 5 μg. quantities of lindane and pentachlorocyclohexene.

Extraction.—Topically treated flies were rinsed after various intervals of time with ethyl ether. Three rinses of 25 ml. each were pooled and the ether evaporated over a steam bath to about 1 ml., and then the remaining ether was removed at room temperature with a gentle airstream. Ether was chosen because of its low boiling point, thus minimizing chances of loss of lindane by volatization. Estimation of the lindane absorbed was made by subtracting that recovered externally from the amount applied.

After rinsing, the ether was allowed to evaporate and the flies were transferred to the flask to be used for dechlorination. The flies were then homogenized with a glass rod and components of the dechlorination mixture added. A constant ratio of 100 flies, 10 ml. acetic acid, 1 gm. powered zinc and 2 gm. malonic acid were used in

[2] Obtained from Dr. H. D. Orloff, Ethyl Corp., Detroit, Michigan.

all the tests. The reaction mixture was then refluxed for 2 hours in the apparatus described by Schechter & Hornstein. Under such conditions known amounts of lindane and pentachlorocyclohexene can be recovered near theoretical quantities with minimums of 5 μg. for the compounds. Interference from flies is so slight that corrections usually need not be made. As illustrated in figure 1, 100 flies add only a negligible amount of absorbance in the range of 330 to 630 mμ.

Since lindane and pentachlorocyclohexene are slightly volatile, an approximation of the amount of material lost by this route was determined. After treatment with lindane or pentachlorocyclohexene, flies were placed in containers through which air was passed slowly into a fritted glass gas dispersion tube submerged in 10 ml. of glacial acetic acid. The amount of lindane or pentachlorocyclohexene trapped in the acid was then determined after definite intervals of time.

RESULTS AND DISCUSSION.—It can be demonstrated by several methods that a compound identical in its chemical and physical behavior to pentachlorocyclohexene is present internally in resistant flies after penetration of topically applied lindane.

Spectrophotometrically the absorption curve of the chromagen resulting from pentachlorocyclohexene (fig. 1) after dechlorination, nitration, and reaction with methyl ethyl ketone and potassium hydroxide, is identical to the curve of the chromagen obtained from extracts of lindane treated resistant house flies (fig. 2).

The absorption curves obtained from the material present externally on resistant (fig. 2) and susceptible flies at any time after topical treatment with lindane are similar to those of lindane (fig. 1). The curve obtained from internal tissues of lindane treated susceptible flies shows mostly the presence of lindane (fig. 2). Dosage and mortality of these flies at various times after treatment are shown in table 1.

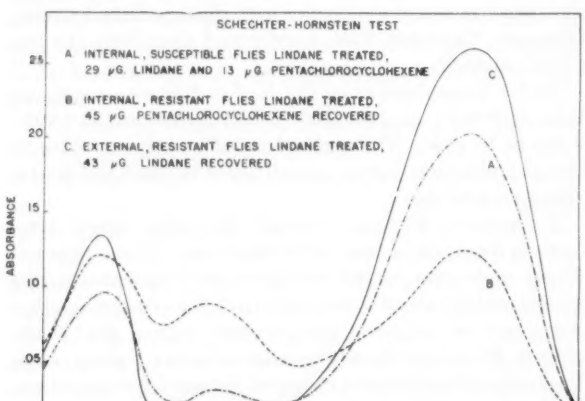

FIG. 2.—Absorption curves of Schechter-Hornstein chromagens obtained from lindane treated house flies. (A) lindane and pentachlorocyclohexene recovered internally from 200 susceptible house flies 8.5 hours after topical application of 0.5 μg. lindane per fly. (B) pentachlorocyclohexene recovered internally and (C) lindane recovered externally from 157 lindane resistant house flies, surviving 4 days on a lindane treated surface and 1 day in a clean flask.

Table 1.—Per cent mortality of lindane resistant and susceptible flies after topical treatment with lindane.

STRAIN OF FLY	DOSAGE IN μg.	TIME IN HOURS	PER CENT DEAD
Susceptible	0.5	1	0
	.5	2	0
	.5	4	25
	.5	8	90
	.5	18	97
Lindane resistant	0.5	1	0
	0.5	2	0
	0.5	4	0
	0.5	8	0
	0.5	17	0
	1.0	1	0
	1.0	2	0
	1.0	4	6
	1.0	8	9
	1.0	17	20
	2.0	1	0
	2.0	2	0
	2.0	4	6
	2.0	8	14
	2.0	16	28
	2.0	24	60

Chromatograms of the Schechter-Hornstein positive products of external extracts of both strains of flies show the presence of only lindane. Extracts of the internal tissues of susceptible flies reveal mostly lindane with traces of pentachlorocyclohexene. From resistant flies, extracts of internal tissues show two well defined spots, one corresponding to lindane, and the other to pentachlorocyclohexene.

Pentachlorocyclohexene appears in the resistant flies shortly after topical treatment with lindane (fig. 4). At the earlier time intervals this compound and lindane account for the applied dosage. As time progresses there is an increasing loss in the total recovered; that is, external lindane plus internal lindane and pentachlorocyclohexene. The quantity of pentachlorocyclohexene does not continue to increase after the first few hours, but this is probably attributable to the metabolism of this compound to products not responding to the Schechter-Hornstein method. Resistant flies treated topically with pentachlorocyclohexene degraded 60.9% of the dose within 24 hours while susceptible flies degraded only 13.7% (table 2).

It is obvious by reference to fig. 4 that flies surviving treatment with lindane will eventually convert the total dosage of lindane to pentachlorocyclohexene and this in turn to products which will not react positively to the

Table 2.—Recovery of pentachlorocyclohexene 24 hours after topical application to 50 resistant and 100 susceptible house flies.[a]

STRAIN OF HOUSE FLY	PER CENT SURVIVAL	PENTACHLOROCYCLOHEXENE Applied μg./fly	Per Cent Recovered: From flies	Volatile	Total	PER CENT NEGATIVE TO SCHECHTER-HORNSTEIN TEST
Susceptible	76	12.75	73.0	13.3	86.3	13.7
Lindane resistant	64	25.50	27.8	11.3	39.1	60.9

[a] Surviving and dead flies analyzed together.

Schechter-Hornstein tests or any of the spot tests used in the paper chromatography. This loss can be only partially attributable to the volatility of lindane and pentachlorocyclohexene. During 24 hours after treatment only about 10% of the 4 μg. dosage of lindane could be accounted for by trapping it from the atmosphere in which the treated flies were retained.

The survivors of lindane-resistant flies, after confinement for 2 to 5 days in a cage coated with lindane, then removed to an uncontaminated cage for 24 hours, may contain pentachlorocyclohexene substantially free from lindane (figure 2). This suggests the possibility of obtaining pentachlorocyclohexene from flies for precise chemical identification.

The rate of absorption of lindane (fig. 3) depends, within certain limits, on the amount applied and on the temperature at which the flies are held. A decrease of either concentration or temperature causes a decrease in absorption. At 35° C. initial absorption was very rapid, approximately 90% of a dosage of 0.5 or 1.0 μg. per fly being absorbed during the first 4 hours after treatment. The rate of absorption decreased as the concentration diminished externally. Flies held at 24° C. absorbed lindane at a slower rate than flies at 35° C. In 4 hours 44, 32, and 23% respectively of 0.5, 2.0, and 4.0 μg. dosages of lindane were absorbed. The rate of absorption of lindane approximates that of DDT. In 4 hours 0.32 μg. of a 0.5 μg. dosage of lindane per fly was absorbed. An almost identical figure is given by Sternburg *et al.* (1950) for the absorption of DDT by DDT-resistant flies.

Both susceptible and resistant flies absorbed a dosage of 0.5 μg. of lindane at approximately the same rate. A change in permeability of the cuticle to lindane does not seem to be a factor in the development of resistance to lindane.

The metabolism of injected dosages of lindane in susceptible and resistant flies was studied by Oppenoorth (1954) using the Schechter-Hornstein method. He found that resistant flies rapidly degraded lindane, with almost complete disappearance of the injected dose within 20 hours. Susceptible flies, on the other hand, degraded lindane more slowly and after 4 hours no further breakdown occurred, and over two-thirds of the dosage was recovered. Oppenoorth questioned whether the more rapid breakdown in resistant flies was the cause of resistance, rather than the result of resistance enabling them to survive longer than susceptible flies at the dosage level used.

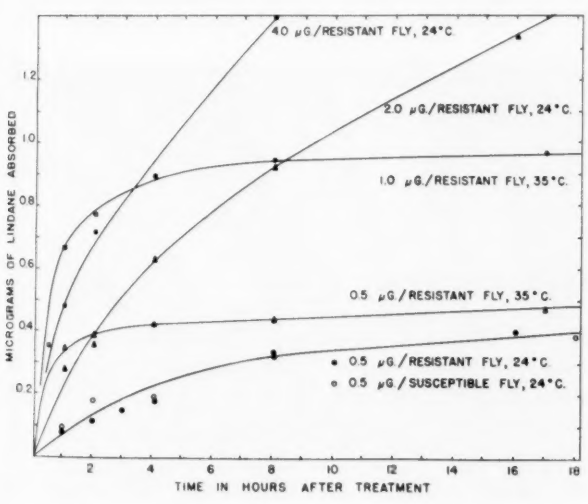
40 μG./RESISTANT FLY, 24°C.
2.0 μG./RESISTANT FLY, 24°C.
1.0 μG./RESISTANT FLY, 35°C.
0.5 μG./RESISTANT FLY, 35°C.
0.5 μG./RESISTANT FLY, 24°C.
0.5 μG./SUSCEPTIBLE FLY, 24°C.
MICROGRAMS OF LINDANE ABSORBED
TIME IN HOURS AFTER TREATMENT

FIG. 3.—Absorption of lindane by susceptible and lindane resistant house flies.

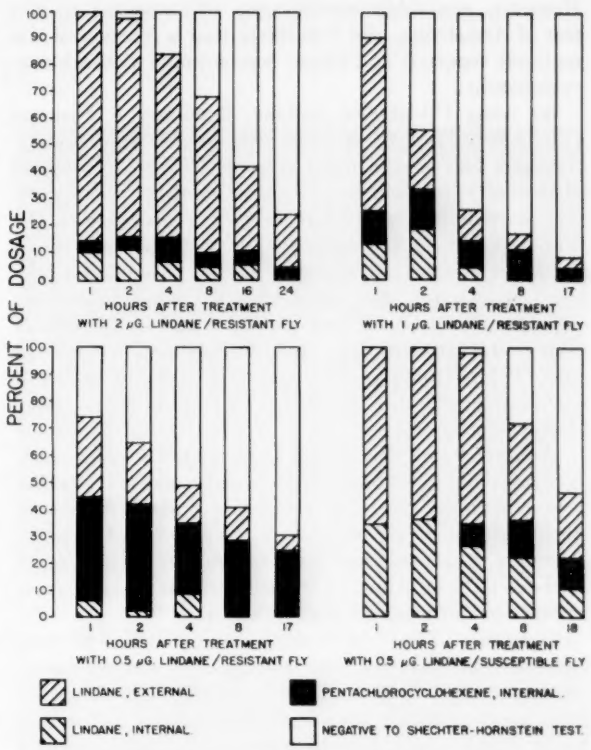
PERCENT OF DOSAGE
HOURS AFTER TREATMENT WITH 2 μG. LINDANE/RESISTANT FLY
HOURS AFTER TREATMENT WITH 1 μG. LINDANE/RESISTANT FLY
HOURS AFTER TREATMENT WITH 0.5 μG. LINDANE/RESISTANT FLY
HOURS AFTER TREATMENT WITH 0.5 μG. LINDANE/SUSCEPTIBLE FLY
LINDANE, EXTERNAL
LINDANE, INTERNAL
PENTACHLOROCYCLOHEXENE, INTERNAL
NEGATIVE TO SHECHTER-HORNSTEIN TEST

FIG. 4.—Recovery from house flies of external and internal lindane, and internal pentachlorocyclohexene at various times after topical application of lindane. See table 1 for per cent dead at each time. Dead and surviving flies were analyzed together.

By using the alpha and delta isomers of BHC, Oppenoorth (1955) showed that his resistant flies were capable of a more rapid degradation of these isomers than was his susceptible strain. From this he concludes that the difference in rate of degradation of BHC may be due to two factors: a greater capacity to degrade BHC by resistant flies, and a lessened capacity to degrade BHC by susceptible flies because of their sensitivity to the toxicant. Part of the product reported by Oppenoorth as lindane or other isomers of BHC in extracts of flies was probably pentachlorocyclohexene, although he could not have known it at the time.

Armstrong *et al.* (1951) developed a different colorimetric analytical method for BHC, based on hydrolysis to trichlorobenzene, nitration to trichlorodinitrobenzene, and reaction of this with aqueous sodium hydroxide to form chlorodinitroresorcinol. Bradbury & Standen (1955) found that during an early step in this method, trichlorobenzene can, if present, be determined independently from lindane. They were unable to demonstrate its presence in the extracts of either susceptible or resistant flies.

However, pentachlorocyclohexene will respond to the test of Armstrong, and it is likely that a fraction of the material reported as lindane was actually pentachlorocyclohexene.

By using C^{14}-labelled lindane, Bradbury & Standen (1955) found up to 50% of the lindane absorbed by resistant flies was converted to a CCl_4-insoluble and H_2O-soluble fraction of unknown identity. Susceptible flies were found to convert a lesser proportion of the absorbed lindane to a water soluble fraction. By chromatographic means they were able to find only one well defined radioactive spot from CCl_4 extracts of both resistant and susceptible flies. This spot had an R_f value of 0.79 to 0.83, identical to that of lindane. Their system used 90% ethanol plus 10% water as the mobile solvent, and vaseline as the immobile solvent on filter paper (Whatman No. 1).

We have found that this system will not separate mixtures of lindane and pentachlorocyclohexene, and that the two compounds behave remarkably alike chromatographically under a wide variety of conditions. It is likely therefore, that the material thought by Bradbury & Standen to be only lindane in their test was also a mixture of lindane and pentachlorocyclohexene.

SUMMARY.—Both susceptible and lindane-tolerant house flies metabolize lindane, the tolerant strain doing so at an accelerated rate. These findings confirm those of Oppenoorth (1954). The rate of absorption of lindane from topical application is approximately the same for the two strains. The enzyme, DDT-dehydrochlorinase, is not involved in the metabolism of lindane. However, pentachlorocyclohexene appears to be an intermediate in the metabolic fate of lindane in house flies. From 0.5 to 2 hours after treatment, recovery of the theoretical quantity of lindane may be accounted for as lindane and pentachlorocyclohexene. At later intervals the quantity of pentachlorocyclohexene does not increase, but the amount of lindane accountable for by the Schechter-Hornstein method decreases. This indicates that pentachlorocyclohexene is an intermediate in the metabolism of lindane. Both strains of flies can readily metabolize pentachlorocyclohexene to products which do not respond to the Schechter-Hornstein test.

Relatively large amounts of pentachlorocyclohexene, substantially free from lindane, may be obtained from the survivors of lindane-tolerant flies exposed for 4 days in a cage heavily coated with lindane. After the exposure, the flies are held for an additional day in a clean cage. Evidence for pentachlorocyclohexene as an intermediate in the metabolism of lindane is based upon spectrophotometric, chromatographic, and colorimetric techniques in which comparisons were made with the product extracted from the flies and the known compound.

ACKNOWLEDGEMENT: The authors acknowledge support of this work by the Rockefeller Foundation.

REFERENCES CITED

Armstrong, G., F. R. Bradbury, and H. Standen. 1951. The penetration of the insect cuticle by isomers of benzene hexachloride. Ann. Appl. Biol. **38:** 555–66.

Bradbury, F. R., and H. Standen. 1955. The fate of gamma benzene hexachloride in normal and resistant houseflies. Jour. Sci. Food Agric. **6**(2): 90–99.

Busvine, J. R. 1954. Houseflies resistant to a group of chlorinated hydrocarbon insecticides. Nature **174:** 783–5.

Decker, G. C., and W. N. Bruce. 1952. House fly resistance to chemicals. Amer. Jour. Trop. Med. Hyg. **1:** 395–403.

Mitchell, L. C. 1952. Separation and identification of chlorinated organic pesticides. Jour. Assoc. Off. Agr. Chem. **35:** 920–7.

Oppenoorth, F. J. 1954. Metabolism of gamma benzene hexachloride in susceptible and resistant houseflies. Nature **173:** 1001–2.

Oppenoorth, F. J. 1955. Differences between rates of metabolism of benzene hexachloride in resistant and susceptible houseflies. Nature **175:** 124–5.

Schechter, M. S., and I. Hornstein. 1952. Colorimetric determination of benzene-hexachloride. Anal. Chem. **24:** 544–8.

Sternburg, J., C. W. Kearns, and W. N. Bruce. 1950. Absorption and metabolism of DDT by resistant and susceptible house flies. Jour. Econ. Ent. **43:** 214–9.

Sternburg, J., C. W. Kearns and H. Moorefield. 1954. DDT-dehydrochlorinase, an enzyme found in DDT-resistant flies. Jour. Agric. Food Chem. **2:** 1125–30.

THOMAS SAY FOUNDATION, VOLUME II

A supply of Volume II, *Plecoptera or Stoneflies of North America*, by J. G. Needham and P. W. Claassen has recently been located. This makes all five volumes available that have been published by the Foundation. Copies of Volume II sell for $5.00 postpaid in North America and U. S. Possessions, and for $5.50 elsewhere.

SCIENTIFIC NOTES

The Tolerance of the Drywood Termite, *Kalotermes minor* Hagen, to Desiccation[1]

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The ability of the common drywood termite, *Kalotermes minor* Hagen, to exist under arid conditions has long been recognized. In the field they are found infesting the dry, dead branches of trees and the dry logs and litter of wooded areas. In and around populated areas the drywood termite occurs in buildings and requires no contact with the soil.

While the drywood termite is known to frequent desiccated wood, it does not necessarily require a dry environment. Observations indicate a preference for a more moist environment, but the amazing ability of this insect to adapt itself to extremes of humidity and aridity makes it a successful species under all conditions. During the winter and late spring, large populations of drywood termites were found in logs in contact with moist soil. These were full-bodied, turgid specimens and were used for the experiments described in this paper. These same termites, during the late summer and fall, when the logs and soil beneath are dry, are much smaller in size because of water loss, but still active and healthy. Laboratory experiments have substantiated these field observations. Termites have been maintained for long periods under normal room conditions, while others have existed for an even longer period under an artificial, moisture-saturated atmospheric condition. Again those living in a humid environment are always long, full-bodied and turgid (see Fig. 1).

The drywood termite will utilize a carton of its own manufacture to establish and seal in moisture when it is needed in situations in which the external environment is dry. The moisture content of each termite contributes toward the over-all requirement of the entire colony. An interesting observation that illustrates the need of close body content to conserve and exchange moisture when it is needed is demonstrated when termites are removed from wood and air-blown to clean and separate them from frass and debris, thus exposing them to radically different outside conditions. The continuous air stream and exposure to sudden dryness has its effect upon them. These termites, when placed under glass in termite observation houses, will quickly huddle, tightly packed together, for a day or two

FIG. 1.—Turgid drywood termites feeding on damp wood under saturated atmospheric condition.

FIG. 2.—Voluntary "packing" of drywood termites to conserve moisture after being subjected to excessive aridity.

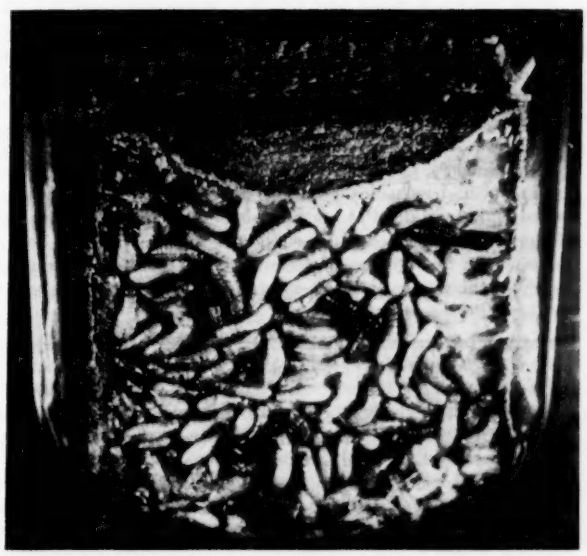

FIG. 3.—Carton canopy built from wood to glass by drywood termites to seal in moisture.

until such time as the gradual sealing of glass to wood is accomplished (Fig. 2). Only then will the termites separate and take on normal colonial activities.

Drywood termites can be induced to build large amounts of protective carton to seal in and retain moisture. This can be demonstrated by placing a solid block of dry wood in the near-center of a glass beaker. Termites poured onto the wood block will lose little time in building canopies and extending the sides of the carton to join the wood with the glass and thus provide the required seal to assure an optimum environmental condition (Fig. 3).

When one considers the extent of heat and dryness to be found in attic areas during the summer in southern California alone, the maximum dry heat tolerance of drywood termites becomes a

[1] Accepted for publication February 7, 1956.

matter of interesting speculation. Information indicates the species can exist in wood with as little as 2½ to 3 % moisture content. With attic areas in some buildings reaching a summer temperature of as high as 150° F., it would seem a considerable amount of sealing would be necessary to sustain life under these extreme conditions. These temperatures and the resulting drying of wood in which the termites dwell seldom occur suddenly. The termites are almost always capable of adjusting themselves to any changes, and thus survive the heat spell. Then again it must be considered that through adequate ventilation enough moisture will enter the vents and be absorbed by the attic timbers during the night and early morning hours, when the air is apt to contain moisture. This, plus the ability of the termites to penetrate deeply into their galleries and provide their own microclimates, will enable them to survive the high temperatures and resulting conditions.

On the other hand, laboratory experiments have proven that, in spite of the great powers of adaptability of the drywood termite, a sudden change in temperature can be fatal. We know that drywood termites can exist under a 150 ° F. attic temperature, but these same termites will expire at 130.5° F. when exposed to infrared heat in the laboratory when they have no opportunity to seek the protection of sealed areas. However, their ability to withstand extreme dryness is another matter. In an effort to determine the maximum tolerance of the drywood termite to extreme dryness, a long-term laboratory experiment was conducted as follows.

Four blocks of white pine 2×3×¾ inches were selected. A cavity 1½ inches in diameter and 3/16 inch deep was carved out of each block to provide a cell in which drywood termites could be placed. One hundred and twenty termites were placed in each cell. Thin plates of clear glass were cut to be placed over the termites within the cell to enable complete observation at all times. Two of the blocks were kiln dried before adding the termites, quickly weighed, and plunged instantly into a desiccator jar containing oven-dried silica gel. The remaining two blocks were sealed and maintained at normal room conditions. The purpose of the experiment was to determine the differences in the reactions of the four groups of termites. The successive losses in weight of the groups of termites kept in the desiccator are shown by the following data:

DATE	WEIGHT OF WOOD AND TERMITES (GRAMS)
Block A	
5- 9-55[a]	37.10
5-21-55	36.62
6- 7-55	36.44
8- 3-55	36.25
9- 7-55	36.10
10-24-55	36.10
1- 9-56	36.10
Block B	
5- 9-55[a]	37.40
5-15-55	37.05
5-22-55	36.82
6- 7-55	36.55
8- 3-55	36.36
10-24-55	36.30
11-10-55	36.27

[a] Date infested wood placed in desiccator.

On August 3, 1955 about half of the termites had died in each cell. All were dead on November 10, 1955 in Block B, but in Block A there remained one alive, although obviously extremely desiccated, on January 9, 1956. This termite was provided with free moisture and was seen to imbibe water for about 1 minute, resulting in an obviously increased turgidity. This termite appeared to be normal in appearance and activity in about 24 hours. It has been kept under conditions of saturated atmosphere and appears to be normal in all respects at the date of this writing (January 17, 1956).

In one of the desiccated blocks of wood not placed in the desiccator, but kept in the laboratory room, all termites were dead on December 1, 1955. In the other, three were alive on January 9, 1956. As can be seen from these data, once the wood was desiccated and the termites were sealed into their cells, they fared about as well when the infested wood was placed in a desiccator as when the infested wood was left under ordinary room conditions.

REFERENCES CITED

Gunn, J. W. 1952. Purdue Conference Report. Pest Control **20**(4): 28.

Harvey, P. A. 1934. Life history of *Kalotermes minor*. *In* Termites and Termite Control. Univ. Calif. Press.

Hunt, R. W. 1949. The common drywood termite as a pest. Jour. Econ. Ent. **42**(6): 959–62.

Light, S. F. 1934. Drywood termites, their classification and distribution. *In* Termites and Termite Control. Univ. of Calif. Press.

Pence, R. J. 1955. Easy-to-build Termite Houses. Pest Control **20**(2): 28–30.

Effects of Iodoacetic Acid on the Coenzyme A Content of the Muscle of the American Cockroach[1]

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Some findings on the coenzyme A content of the muscle of the American cockroach, *Periplaneta americana* (L.), have been reported in an earlier paper (Bettini & Boccacci, in press). In view of the fact that the activity of CoA is bound to the —SH group contained in the molecule it was decided to include in a series of investigations on the insecticidal activity of iodo-, bromo-, and chloroacetic acids (halogen-containing alkylating agents) (Bettini & Boccacci 1951, 1954, 1955, Bettini *et al.* 1955) a study of the action of iodoacetic acid on CoA *in vivo*.

Nachmansohn & Wilson (1951) had previously reported that CoA is inactivated *in vitro* by iodoacetic acid by the reaction

$$CoA—SH + ICH_2COOH = CoA—SCH_2COOH + HI$$

Adult males of *P. americana* were used as biological material. CoA was estimated in the metathoracic coxa (one side only) *in toto;* after iodoacetic acid inoculation[2] the estimation was then repeated on the metathoracic coxa of the opposite side. For the techniques used in preparing the material and in inoculation the reader is referred to an earlier paper (Bettini & Boccacci 1955). The coxae were removed about 2 hours after inoculation in the case of specimens treated with 180 μg. of iodoacetic acid (2 LD_{50}) and about 1 hour after inoculation in the case of specimens treated with 900 μg. (10 LD_{50}). In both cases the cockroaches were knocked down but were not yet dead at the moment of removal.

For CoA estimation the following procedure was followed:

a) Specimens treated with 180 μg. of iodoacetic acid.

1 gm. of material was kept in the bottom of a test tube in a boiling water bath for 10 min., and then transferred to a cooled glass mortar at 0° and ground up with quartz sand after addition of 5 ml. of iced water. The extract was left at 0° for 15 min. and then centrifuged in a refrigerated centrifuge.

b) *Specimens treated with 900 μg. of iodoacetic acid.*

PAID PAPER.
[1] Accepted for publication April 23, 1956.
[2] Iodoacetic acid was always inoculated in the form of the sodium salt in physiological saline at pH 7.5.

Working at 0°, 1 gm. of material, after addition of 5 ml. of water, was ground up and then deproteinized according to Awapara's (1948) method.

In both cases, CoA is estimated by the "acetylation of sulfanilamide" method, as reported by Novelli (1955), on 0.5 ml. of the deproteinized extract.

Two different methods of deproteinization had to be followed because the first method, although simpler to carry out, would have allowed the excess of iodoacetic acid present in the tissues of specimens treated with 900 μg. to react with the CoA during the treatment on a boiling water bath. In previous work with bromoacetic acid labelled with C^{14}, while no bromoacetic acid remained in circulation in specimens treated with 180 μg., since it had reacted completely 45 minutes after inoculation (Bettini *et al.* 1955), in specimens treated with 900 μg. considerable quantities of bromoacetic acid remained in circulation even at the moment of death (Bettini & Boccacci 1956).

It has already been shown by Barron (1951) that iodo- and bromoacetic acids possess equal reactivity to substances containing an —SH group (cisteine).

Table 1.—Coenzyme A content in units/gm. of fresh material of *Periplaneta americana*.

Test No.	Normal Coxae	Treated Coxae: 180 μg. of ICH_2COOH per Specimen	Treated Coxae: 900 μg. of ICH_2COOH per Specimen
1	11.4	9.5	9.2
2	11.5	9.4	—

As will be seen from table 1, the reductions of the CoA content of the muscle produced by doses of 180 μg. and 900 μg. of iodoacetic acid, corresponding to 2 and 10 LD_{50}, are 16 and 19%, respectively.

It has been shown in an earlier paper (Bettini & Boccacci 1955) that a dose of 900 μg. per specimen completely inhibits the triosephosphate dehydrogenase activity of the coxal muscles of *P. americana;* so that CoA inhibition very probably has less importance in iodoacetic acid intoxication than triosephosphate dehydrogenase inhibition.

References Cited

Awapara, J. 1948. Application of paper chromatography to the estimation of free amino acids in tissues. Arch. Biochem. **19:** 172–3.

Barron, E. S. 1951. Thiol groups of biological importance. Advances Enzymol. **11:** 201–66.

Bettini, S., and M. Boccacci. 1952. Sostanze alogenate alchilanti come insetticidi di contatto per le mosche resistenti. Riv. Parassitol. **13**(2): 165–7.

Bettini, S. and M. Boccacci. 1954. Localizzazione e distribuzione della succinodeidrogenasi nel muscolo di insetti. Rend. Ist. Super. Sanità **17:** 188–91.

Bettini, S. and M. Boccacci. 1955. Azione tossica degli acidi iodo- e cloroacetico sugli insetti. Inibizione della triosofosfatodeidrogenasi Riv. Parassitol. **16**(1): 13–29.

Boccacci, M., and S. Bettini. 1956. Co-enzyme A content in the American cockroach (*Periplaneta americana* L.) and the house fly (*Musca domestica* L.). (In press.)

Bettini, S., M. Boccacci and C. Rossi. 1955. Destino dell'acido bromoacetico marcato con C^{14} inoculato in *Periplaneta americana*. Riv. Parassitol. **16**(2): 103–12.

Nachmansohn, D., and J. B. Wilson. 1951. The enzymic hydrolysis and synthesis of acetylcholine. Advances Enzymol. **12:** 259–339.

Novelli, D. 1955. Methods of biochemical analysis, Vol. II, p. 201.

A Laboratory Method for Biological Testing of Insecticide Sprays Used in Forest Insect Control[1]

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In research on the distribution of aerial sprays for the control of forest insect pests at the Beltsville, Md., Forest Insect Laboratory, it was believed advisable to expose insects to the deposits obtained. In this work, therefore, tests were designed to give an index to the biological effectiveness of the deposit and atomization. The season for conducting tests of biological effectiveness of sprays under natural conditions in the field is short, and infestations of suitable insects are sometimes scarce. As a result, reared insects that can be made available any time in any quantity are most suitable for the studies. The American cockroach, *Periplaneta americana* (L.), was selected for the method described here. The method is used in experiments conducted with test insects (1) by placing them on foliage sprayed by airplane and (2) by placing them on glass and foliage sprayed by a laboratory spray chamber (Eaton & Secrest 1948).

Selection of Test Insect.—When this work was initiated, a search was made for a test insect species that (1) could be reared easily at all seasons and in large numbers, (2) was small enough to confine in numbers in a petri dish, yet large enough to count easily, and (3) was physically hardy and somewhat resistant to DDT. A forest insect would have been desirable for this testing work but, unfortunately, difficulties involved in collecting, rearing, and handling such insects made it necessary to use a substitute species. In searching for the most satisfactory species for testing, the confused flour beetle, several different species of cockroaches, a sawfly, and the European corn borer were investigated. One of these, the confused flour bettle, *Tribolium confusum* Duv., was used for tests during one season. Now, the American cockroach, *Periplaneta americana* (L.), which is less susceptible to DDT and more closely related to destructive forest defoliators in its reaction to DDT, is used entirely. The sawfly and European corn borer were unsatisfactory because of rearing difficulties.

Description of Method.—In the tests over open ground, spray is discharged from an airplane flying across a line of sampling stations sufficiently long to permit the sampling of the entire spray swath. Spray falling beneath the airplane is collected in petri dishes 100 mm. in diameter. Dish lids are then replaced and the dishes brought into the laboratory, where 15 three-week-old cockroach nymphs are placed in each dish, usually during the same day. Tests here show that dishes containing deposits can be held for 1 week before the introduction of cockroaches without affecting results. As a check, 15 cockroaches are also placed in an unsprayed dish. Pellets made of ground dog food moistened with a glycerine-water mixture (50–50) pressed into a flat-bottomed dish to a depth of about 3 mm. served as food for the cockroaches. The use of the moistened pellets makes it unnecessary to provide water, and the glycerine serves as a mold inhibitor. Throughout the tests the dishes are protected from direct sunlight, as it has been observed that when test dishes were exposed to sunlight mortality was almost 100%, regardless of other factors.

Mortality counts are made after 72 hours of continuous exposure to the deposit. Longer exposure gives little or no added mortality from the DDT, and shorter exposure does not insure the maximum effect. Only two categories, dead and alive, are used in the mortality counts. Those insects on their backs or showing symptoms of DDT poisoning are counted as dead.

In field tests, flat glass plates are used to catch the spray. Petri dishes are not used because their raised sides interfere with spray

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[2] Grateful acknowledgement is made of the help of C. B. Eaton who was mainly responsible for the initiation of work leading to the method described.

deposition, especially when there is any lateral air movement. After exposure to the falling spray, the glass plates are brought into the laboratory. Cockroaches are then confined on them by ground-bottom lantern globes. The area enclosed by a globe is the same as the area in a petri dish section. Each globe is covered with a glass plate to maintain conditions as nearly like the covered petri dishes as possible (Fig. 1).

Biological tests of deposits on foliage are conducted in essentially the same manner as are those on glass. Samples consisting of 3-inch twig tips of conifers, or of entire leaves of broad-leaved plants, are collected and brought into the laboratory where they are placed in covered glass evaporation dishes or finger bowls and infested with 15 cockroach nymphs (Fig. 2). Untreated checks are treated in the same way. Since most foliage is attractive to the cockroaches, no difficulty is experienced in getting them to come in contact with the deposit. Food is omitted from foliage tests to prevent congregation of cockroaches on the food pellet. Mortality counts are made after 6, rather than 3 days' exposure, since the maximum effect is not evidenced until that time.

Cockroach nymphs can also be tested on sprayed foliage outdoors. In this method the nymphs are confined in sleeve cages made from screen wire, with the seams crimped or soldered. One end of the cage is closed, and the other is extended with cloth so that it may be tied against the stem by means of a draw string. Mortality in this type of cage is comparable to that obtained by indoor tests in dishes. Trees used in these tests may be potted until after spraying, then transplanted in the ground, and the biological tests made.

The biological tests of deposits obtained from a spray chamber are conducted in the same manner as those described above. In tests of spray chamber deposits on glass, however, petri dishes are easier to handle than glass plates and lantern globes. To test the residual effect of deposits on glass that have been subjected to weathering, mortality counts are made after cockroaches have been exposed to the deposit for 6 days. Tests with spray chamber deposits on foliage are conducted as described above for airplane spray deposits on foliage.

Sprayed tips of twigs are placed outdoors in vials to expose deposits on foliage to the effects of weathering. These twigs remain fresh for several months to temperatures above freezing, if the water in the vials is replenished two or three times a week.

The procedures for biological testing wherein cockroaches are used as test animals should be applicable to other insects also. They have been used successfully in studies of the spruce budworm *Choristoneura fumiferana* (Clem.), the white pine weevil, *Pissodes strobi* (Peck), and the eastern tent caterpillar, *Malacosoma americanum* (F.). Where larvae are used, however, it is necessary to confine them by barriers. The procedures should also be useful in testing other insecticides and formulations.

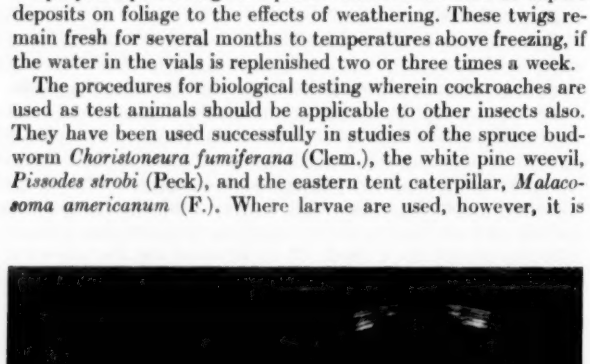

FIG 1.—Test units: left—petri dish; right—lantern globe over sprayed plate.

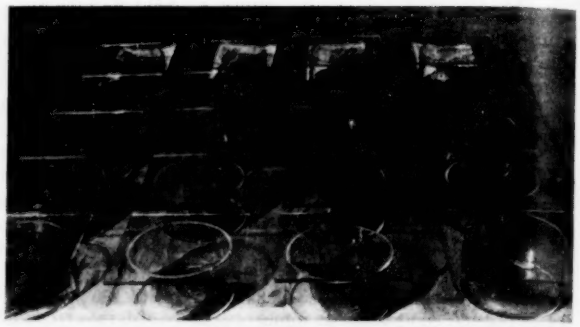

FIG. 2.—Sprayed foliage in evaporating dishes.

SUMMARY.—To facilitate the valuation of aerial spraying equipment, a biological test that utilized the American cockroach, *Periplaneta americana* (L.), as a test animal, was designed. Basically, the test consists of exposing cockroaches to deposits of insecticide in petri dishes or on glass plates. The method, originally used in evaluating flight tests over open ground, can also be used to test deposits applied by a spray chamber in the laboratory. With modification the method can be used for studies of foliage sprayed in either the field or laboratory. In studies of the latter type, cockroaches are exposed to sprayed twig tips from conifers, or to leaves from broad-leaved plants in evaporating dishes. Maximum mortality is evidenced after 3 to 6 days' exposure to deposits. The test has been used in studies of dosage-mortality relationship, atomization, weathering of deposits, and bio-assay in formulation testing. The same procedures should be useful in making similar studies of certain other insects.

REFERENCES CITED

Eaton, C. B., and J. P. Secrest. 1951. A laboratory spray chamber for producing deposits of limited drop size. U. S. Bur. of Ent. and Plant Quar. **ET-293.** 6 pp., illus. (Processed.)

A Study of Insecticide Resistance in House Flies of Japan and Okinawa[1]

G. W. BYERS,[2] C. M. WHEELER[3] and T. E. BLAKESLEE[4]

Resistance of the house fly, *Musca domestica* L., to DDT has been noted and resistance to lindane has been suspected by a number of investigators in Japan. However, this insect's degree of resistance to DDT and other chlorinated hydrocarbons and its tolerance of the organic phosphates have received minor attention (Sasa 1955). DDT has been used extensively as a house fly control agent in Japan and Okinawa since 1945. More recently lindane and BHC have been adopted in areas of Japan where DDT was no longer giving effective control, while malathion has been used only in limited field studies.

Difficulty in controlling house flies with DDT in the vicinity of a military installation in Yokohama led to the adoption of lindane in April, 1954 (Johnson 1954). BHC was selected as a house fly control agent at the Tokyo Municipal Garbage Dump in August, 1954 when the city health officials noted that DDT

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Table 1.—Toxicities of four insecticides to five house fly strains.

SOURCE OF FLY STRAIN	PREVIOUS HISTORY OF INSECTICIDE APPLICATION	M_{50}-DOSAGE OF INSECTICIDE CAUSING 50% KILL[a]			
		DDT	Lindane	Dieldrin	Malathiln
Municipal Garbage Dump, Tokyo, Japan	DDT & BHC	13.1	2.9	0.21	0.33
Cow Stable, Yokohama, Japan	DDT & Lindane	12.9	2.1	0.06	0.44
Garbage Dump, Yokohama, Japan	DDT	8.2	0.30	0.24	0.66
Johnson Air Force Base, Japan	DDT	8.2	0.25	0.22	0.28
Kadena Air Force Base, Okinawa	DDT	12.2	0.007	0.01	0.23

[a] Micrograms per 4 microliters of solution.

was no longer effective. Malathion was substituted 3 months later when it became apparent that BHC also had ceased to give satisfactory control (Yamaguchi 1954). Ojima & Nagae (1955) found Chlorthion more toxic to house flies than BHC but less toxic than parathion. Yamazaki & Ishii (1955) studied the relative effects of DDT and methoxyclor on a strain of DDT-resistant flies and found methoxychlor to be more toxic.

The present study was undertaken to determine, for purposes of comparison with future M-50 values, the degree of resistance in house flies to DDT, lindane, dieldrin and malathion in Japan and Okinawa.

METHOD.—Laboratory strains were established during the fall of 1954 from each of five areas where the history of insecticidal application was clearly known. Two areas had received lindane treatment during 1954 after DDT had failed to control house flies: the Tokyo garbage dump, and a cow stable adjacent to a military dependent housing area in Yokohama. Three areas had been treated only with DDT: a municipal garbage dump in Yokohama; Johnson Air Force Base, 25 miles northwest of Tokyo; and Kadena Air Force Base, Okinawa.

Larvae were cultured in a mixture of CSMA larval medium, water, molasses and yeast, as described in the Peet-Grady method (Anon. 1953). A constant ratio of volume of viable eggs to volume of medium was maintained in seeding the cultures. Adult flies were fed daily on whole milk.

Female flies, 3 to 5 days old, were used in all tests, as males and females have been found not to react equally to insecticides. The flies were anesthetized with carbon dioxide. Each fly was given a dose of 4 microliters of acetone containing a graded dilution of toxicant, the dose being applied to the dorsal thoracic surfaces from a 0.25-ml., micrometer-actuated tuberculin syringe, as described by Trevan (1922). Acetone alone was used in all the control groups. A minimum of three replicates of 10 flies were treated at each of the four dosage levels used in computing the M-50, for each insecticide. Treated flies were transferred to 2-gallon glass animal jars equipped with stockinette sleeves, where they were retained for 24 hours at a temperature of 27° C. ± 2° and provided with food and uniform humidity. At the end of this period mortality was recorded and the M-50 values computed by the Thompson method (Glasser 1950, Wadsworth 1947).

RESULTS.—Test results are presented in table 1 and represent averages of at least three complete test series. All five strains were moderately resistant to DDT. Those from the Tokyo City Dump, Yokohama stable and Kadena Air Force Base were approximately 35% more resistant than the strains from the Yokohama Garbage Dump and Johnson Air Force Base. Flies from all colonies were highly susceptible to dieldrin and malathion. The two strains which had been previously exposed to lindane (or BHC) for a period of less than a year were approximately 10 times more resistant to this material than were the three strains which had not been exposed.

SUMMARY.—The susceptibilities of five strains of the adult house fly, *Musca domestica* L., to four insecticides were determined by topical application. All strains, four from Japan and one from Okinawa, were moderately resistant to DDT and highly susceptible to dieldrin and malathion. Two strains, one which had been exposed to lindane and the other which had been exposed to BHC for a period of less than a year, were approximately 10 times as resistant to lindane as were the other three strains which had received no exposure to this material.

REFERENCES CITED

Anonymous. 1953. Peet-Grady Method. Soap Blue Book.: 224–5. MacNair-Dorland Co., New York, N. Y. 242 pp.

Glasser, O. 1950. Medical Physics. Vol. II. Year Book Publishers, Chicago. 1227 pp.

Johnson, M. S. 1954. Personal communication of September 30, 1954.

Ojima, K., and H. Nagae. 1955. Comparison of toxicities of parathion, chlorothion and BHC to houseflies employing topical application. Proc. Annual Congress of the Societies of Applied Zoology and Applied Entomology.: **20.**

Sasa, M. 1955. Personal communication of October 14, 1955.

Trevan, J. W. 1922. An apparatus for measuring small quantities of fluids. Lancet **202:** 786.

Wadsworth, A. B. 1947. Standard methods of the division of laboratories and research of the New York State Department of Public Health. Williams and Wilkins Company.

Yamaguchi, Y. 1954. Personal communication of August 1, 1954.

Yamazaki, T., and T. Ishii. 1955. DDT resistent houseflies and DDT analogues. Proc. Annual Congress of the Societies of Applied Zoology and Applied Entomology.: **20.**

Dieldrin and Endrin for Control of DDT-Resistant Potato Flea Beetles[1]

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Insecticide tests for the control of DDT-resistant potato flea beetles, *Epitrix cucumeris* (Harr.), in Connecticut during 1953 and 1954 have been reported by Kring (1955). The comparisons between insecticides used in these tests were not entirely satisfactory, because of the severe reduction of the population on all the test plots including untreated checks, soon after the first application. Several changes were made in the 1955 tests in an attempt to obtain more dependable results. The number of replicates was increased from 3 to 4. The number of materials tested was reduced from 11 to 3. The number of check plots was increased from 4 to 13 per replicate. Untreated checks in adjoining plots were paired rather than randomized to decrease the loss of

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[2] The assistance of Mr. A. DeCaprio is gratefully acknowledged.

flea beetles from checks by migration to treated plots. The highest dosage of all materials was decreased to the estimated lowest effective dosage, except in the case of DDT, which was not controlling flea beetles at normal dosage. Dilution in the dosage series was increased from 2 to 3 times.

The tractor-drawn boom sprayer used in 1955 and 1954 was that described by Woodruff & Turner (1946).

Katahdin potatoes were planted on May 3, 1955, at Windsor, Conn. Insecticide applications were made on June 1 and 14 and on July 12 and 26. On June 21 counts were made of the protection given by the first two treatments. In these counts the terminal leaflet of the fourth leaf down from the top of the plant was picked from 15 plants in each plot. The number of feeding punctures in each surface of the leaflet was recorded. The flea beetle damage was that done by the overwintering adults.

Potato plants in several portions of this field were severely injured or killed by August 9 by drought. It was not possible to take further data.

The materials, dosage levels and the estimates of effectiveness of these treatments are given in table. 1. Good response to

Table 1.—Control of potato flea beetle in 1955. Windsor, Conn.

W.P. Insecticide	Pounds Toxicant per Acre	Potato Flea Beetle	
		Feeding Punctures	Per Cent Reduction
Dieldrin, 50%	0.250	26	96.9
	.083	79	90.7
	.028	112	86.9
	.009	420	50.7
Endrin, 25%	.250	39	95.4
	.083	193	77.3
	.028	203	76.2
	.009	379	55.5
DDT, 50%	2.000	178	79.1
	.667	316	62.9
	.222	366	57.0
	.074	766	10.1
Untreated Check		852.3	

the different dosages tested is shown. This test indicates that the effective dosage of endrin is 0.25 pound per acre. Dieldrin is effective at rates considerably lower and gave satisfactory protection from flea beetles at a rate of 0.08 pound per acre. To obtain equal protection with DDT a considerable increase in dosage over the 2 pounds of actual toxicant used would be necessary. The control of flea beetles following application of 2 pounds of DDT was about the same as that given by 0.08 pound of endrin and 0.02 pound of dieldrin.

Although it is unsafe to compare results obatined in different seasons, particularly where the experimental design and experimenters have been changed, it is interesting to note that 1 pound of DDT at Mt. Carmel, Conn. in 1946 (Woodruff & Turner 1946) gave equal or better protection than that obtained with 2 pounds at Windsor in 1955. Again at Mt. Carmel in 1948 and in 1953 amounts of DDT as low as 0.25 pound gave equal or better protection than that obtained with 2 pounds of DDT at Windsor in 1955.

In table 2 the results obtained at Windsor, Conn. in 1954 and 1955 for the same insecticides are compared. The changes in design made in the field test in 1955 gave more suitable dosage responses for the three insecticides tested than had been previously obtained in 1954. Obviously this design provided a better comparison of insecticides.

A separate field test was made on Kennebec potatoes planted May 17, 1955, at Mt. Carmel, Conn. In this test endrin was applied at a rate equivalent to 1 pound of wettable powder, containing 25% active ingredient, to 100 gallons of water per acre. At this rate endrin gave protection from potato flea beetle damage to the potato foliage similar to that obtained at Windsor in 1955.

Table 2.—Control of potato flea beetles in 1954 and 1955. Windsor, Conn.

Insecticide	Pounds Toxicant	Per Cent Reduction	
		1954[a]	1955
DDT	2.00		79.1
	1.00	55.6	
	.67		62.9
	.50	0.0	
	.25	66.4	
	.22		57.0
	.07		10.1
Dieldrin	1.000	86.0	
	.500	70.8	
	.250	65.1	96.9
	.083		90.7
	.028		86.9
	.009		50.7
Endrin	.500	85.8	
	.250	85.2	95.4
	.125	71.3	
	.083		77.3
	.028		76.2
	.009		55.5
Untreated Check		—	—

[a] Data from Kring (1955).

Summary and Conclusions.—Good response to different dosages of DDT, dieldrin and endrin was obtained from field applications of these insecticides for control of DDT-resistant potato flea beetles. These responses were much better than those obtained in field tests in 1954 at the same location with the same sprayer and operators. This improvement in response was obtained by altering the design of the field experiment to increase the amount of untreated area and by decreasing the amount and numbers of effective materials. It is believed that these changes allowed for longer and greater survival of potato flea beetles in the test area.

Dieldrin was effective against the potato flea beetle at 0.08 pound per acre. Endrin was effective against the same insect in two separate field tests at 0.25 pound. The amount of DDT that would be necessary to give protection equal to that obtained with these amounts of either dieldrin or endrin would be extremely high.

References Cited

Kring, James B. 1955. Control of DDT-resistant potato flea beetles. Conn. Agr. Expt. Cir. **193.**

Woodruff, N., and N. Turner. 1946. Control of potato pests with new organic insecticides. Conn. Agr. Expt. Bull. **512:** 60–67.

Methods for the Mass Rearing of the Pink Bollworm[1]

ERMA S. VANDERZANT[2,3], RAYMOND REISER[3], and E. E. IVY[2,4]

The pink bollworm, *Pectinophora gossypiella* (Saund.), can be reared in the greenhouse on cotton plants or on cotton bolls that are kept moist in water or sand. However, because of the length of time required to grow a cotton plant, as well as the limited number of other host plants, this method is impractical. Artificial rearing techniques are complicated by the extreme cannibalism of the larvae. A method of avoiding all these difficulties is needed so that a supply of pink bollworms is available for laboratory studies during the winter when no field specimens can be obtained.

Two rearing methods that are relatively easy to perform and reasonably successful are described in this paper. In one method either immature or sprouted seeds of beans and peas are used under aseptic conditions, and in the other sprouted cottonseed is used under conditions that permit a minimum interference by contaminating microörganisms.

ASEPTIC METHOD FOR PEAS AND BEANS.—Immature peas were purchased at a local grocery and needed no previous preparation except removal from their pods. To prepare sprouted seeds with radicles $\frac{1}{4}$ to $\frac{1}{2}$ inch long, seeds were soaked in water for 6 hours, and then drained and placed in covered dishes to sprout. About 40 hours were needed for sprouting pea seed, although the time varied with the batch.

The immature or sprouted seeds were put into 500-ml. Erlenmeyer flasks having a layer of absorbent cotton on the bottom to absorb the water formed during the autoclaving process. In several experiments each seed was individually wrapped to keep the larvae apart, but this was found to be unnecessary. The flasks were plugged with nonabsorbent cotton and autoclaved at 15 pounds' pressure for 15 minutes for immature seeds and for 20 to 25 minutes for sprouted seeds. Eggs used for inoculation were collected and washed as previously described (Vanderzant & Reiser 1956). Upon hatching, larvae were placed in the flasks, which were kept in an incubator at 84°F. Larvae entered the seeds and did not reappear until they reached the fourth instar. Larvae and pupae were removed on the day the first pupa was observed or shortly thereafter, so that they could be counted. Larvae were then placed in wet cotton in a petri dish to pupate and the pupae were weighed.

As shown in table 1, the largest and most rapidly developing larvae were obtained from sprouted peas. Larvae pupated as early as 14 days after inoculation, and the pupae had an average weight of about 20 mg., which is comparable to field specimens. Larvae reared on immature peas were smaller, but all pupated within a few days and normal moths emerged. There were approximately equal numbers of males and females. These moths were put in an oviposition box and approximately 900 fertile eggs were deposited by them. The yield of eggs per female appeared to be high, although no precise count was made. No records were kept of eggs produced by moths from other experiments although all moths appeared to be normal.

METHOD FOR COTTONSEED.—Delinted cottonseeds were soaked in water for 6 hours, and then drained and placed in covered dishes to sprout. About 16 to 20 hours were needed for sprouting. They were then placed in a solution containing 2 grams of methylcellulose (Dow Methocel, 400 CPS) and 0.5 gram of *n*-butyl parahydroxybenzoate (Butoben) per 100 ml. of water for about 5 minutes. The excess liquid was drained off, and the seeds were allowed to dry until they were no longer sticky. They were then placed on a thin layer of absorbent cotton in plastic refrigerator dishes, 8×4×3 inches, and inoculated with newly hatched larvae or eggs about ready to hatch. The eggs were not washed, but were left on the screen from the box on which they were deposited. The screen was placed on the surface of the seeds and the dish covered tightly to keep the larvae from crawling out. After the larvae disappeared into the seeds, the covers were removed and not replaced until the fourth-instar larvae began to emerge. If the dishes were tightly covered during the entire larval period, the seeds continued to grow and the growth of molds was encouraged. The dishes were kept on a laboratory bench in a room having a mean temperature about 80° F. and a relative humidity range of 70 to 90%.

Table 1.—Mass rearing of the pink bollworm larvae.

METHOD	NUMBER OF SEEDS IN ONE CONTAINER	NUMBER OF LARVAE	DAY OF FIRST PUPA	AVERAGE WEIGHT OF PUPAE (MILLIGRAMS)
Aseptic:				
Immature green peas	190	30	15	15.1
	120	37	18	
Sprouted peas	240	43	14	18.6
	265	57	14	21.6
Sprouted beans	90	21	17	13.6
		34	17	12.3
		15	16	13.1
Cottonseed	550	52	11	18.1
		43		
		40		
		45		

The unheated cottonseed was quite satisfactory for growth of the larvae. Pupation began 11 days after inoculation, which is comparable to the development of larvae on squares. The coating applied to the seeds was effective for about a week and served two purposes, to prevent drying and to inhibit mold growth. By this time the larvae had matured. It was important also to keep the humidity of the environment high so that the seed would remain moist on the inside and dry on the outside. It is probable that methylcellulose could be replaced by other coating agents and that other mold inhibitors could be used instead of Butoben, but no others have been tried. Although some mold inhibitors are toxic to the larvae, this is not a serious problem because the larvae do not feed on the outside of the seed.

The aseptic method was also tried for sprouted cottonseed. In one experiment seven very small fourth-instar larvae were obtained, but in three other trials none developed beyond the second instar. It was concluded that the autoclaving was destroying one or more of the essential nutrients.

Microbial decomposition within the seeds prevented the rearing of larvae on sprouted peas and beans by the method used for cottonseed.

DISCUSSION.—By both methods approximately 30 to 60 larvae were reared together in one container. The limiting factors seem to be the size of the containers and the number of larvae used for inoculation, since only 50 to 100 larvae were placed in each container. Best results were obtained if the seeds were spread over a large area in a layer not more than $\frac{3}{4}$ inch thick. The number of seeds to place in the rearing containers was determined from the volume occupied by the dry seeds because they were usually introduced from a 100-ml. beaker. The size of the seed, therefore, accounts for the great difference between the number of beans and cottonseed.

Although the pink bollworm is considered to be an obligatory insect, the larvae have been reared in our laboratory on synthetic diets containing no cotton plant products (Vanderzant & Reiser 1956). It is not surprising, therefore, that they can be

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[4] Resigned November 1, 1955.

reared on other seeds. Peas and beans are similar in nutritive value, and in the germination process this value is increased by hydrolysis of protein and carbohydrate and development of enzymes, intermediary metabolites, and vitamins. The nutritive value of cottonseed changes similarly during germination. Cheldelin & Lane (1943) have reported a twofold increase in the B vitamins of cottonseed during a 36-hour germination period.

The rearing of larvae on sprouted peas is the preferred aseptic method for rearing small numbers of larvae for laboratory studies because fresh immature peas are not available the year round and the yields with sprouted beans were not as good. However, the preparation of the seed is time-consuming and great care must be taken to avoid contamination. With improvements the cottonseed method should give better yields. It has the advantage of being easily and rapidly carried out.

References Cited

Cheldelin, V. H., and R. L. Lane. 1943. B vitamins in germinating seeds. Proc. Soc. Expt. Biol. Med. **54**: 53–55.

Vanderzant, Erma S., and Raymond Reiser. 1956. Aseptic rearing of the pink bollworm on synthetic media. Jour. Econ. Ent. **49**(1): 7–10.

The Dilution of Dust Concentrates in Pink Bollworm Control[1]

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More economical control of cotton insects with insecticidal dusts would be possible if smaller amounts of more concentrated dusts could be used in applying the desired amount of toxicants to the plants. A field experiment was conducted at this Station during the summer of 1955 to compare the effectiveness of dilute and concentrated dust applied at an equal amount of active ingredient per acre. A field which had an early infestation of pink bollworm, *Pectinophora gossypiella* (Saund.), was chosen for the location of this experiment. Although records were made of other cotton pests present, none of them were numerous enough to give any indication of the efficiency of the treatments.

Field Plots and Treatments.—A randomized block, split-plot arrangement with five replicates was used for this experiment. The two main plots in each block were treated with different insecticides, and the two split plots in each main plot were treated with different dilutions of the same insecticide. The split plots afforded adjacent comparison between the dilutions of each insecticide. The main plots gave comparisons of the two different insecticidal treatments. No check plots were included, but an outside check plot was left to show the population differences between the treated and untreated parts of the field. Each split plot was 12 rows wide, 108.9 feet long and one-tenth of an acre in size.

The two treatments used were DDT-dieldrin and Bayer 17147. The concentrations of the former were 10% DDT and 1½% dieldrin at the high poundage of 20 pounds per acre, while for the low poundage of 10 pounds per acre, 20% DDT and 3% dieldrin were used. The latter insecticide, Bayer 17147, was used as a 5% dust at the low poundage and as a 2½% dust at the high poundage.

Dusts were applied by means of rotary hand dusters and the amount per acre was controlled by closing or opening the dust-port and by the speed at which the applicator walked. One of the difficulties of this experiment, to apply approximately the same amount of active ingredient at the low and high levels of both insecticides, was successfully resolved (Table 1). Insecticides were applied six times at 7-day intervals from July 2 to August 9. At that time a high percentage of the bolls had opened.

Table 1.—Average pink bollworm infestation and yields of seed cotton at College Station, Texas.

Treatment	Pounds of Dust Applied per Acre	Pounds Active Ingredient per Acre	Per Cent Infested Bolls	No. of Mines per 50 Bolls	Yield	
					Pounds per Plot	Pounds per Acre
DDT-dieldrin						
20%–3%	9.9	2.28	30.6	25.4	59.6	1490
10%–1.5%	20.2	2.32	27.0	23.5	57.6	1450
Bayer 17147						
5%	10.6	0.53	22.4	16.3	66.6	1665
2.5%	20.7	0.52	23.8	20.8	62.0	1550
Outside check	—	—	73.4	112.2	—	—

Records.—Infestation counts were made 1 day previous to each application. Per cent boll weevil punctured squares and per cent cotton bollworm injured squares were recorded in the usual manner until July 15, when squares had disappeared almost completely. Per cent bollworm injured bolls were recorded until July, when no more damage was observed. Pink bollworm infestation was estimated by collecting 50 firm bolls at random from each plot and cutting them in the usual manner to record the number of infested bolls and the number of mines per sample. These counts were made weekly until there were no green bolls left in the field.

Results.—Pink bollworm infestation data and yield are shown in table 1. Analysis of variance showed no significant difference between the dilute and concentrated dusts when the same amount of active ingredient was applied. Bayer 17147 was significantly better than the DDT-dieldrin combination in reducing the numbers of pink bollworm infested bolls. In the outside check the infestation reached 100% and had a seasonal average of 73.4%. There was no significant difference between the dilutions nor between the insecticides in number of pink bollworm mines. The check plot had an average of from 4 to 5 times as many mines as the treated plots. There was no significant difference in yield between the dilute and concentrated dusts nor was the difference between Bayer 17147 and DDT-dieldrin significant.

Results of this test indicated that it may be unnecessary to apply very high poundages of insecticidal dusts to control the pink bollworm if more concentrated dusts are carefully applied. Bayer 17147 gave somewhat better control of the pink bollworm than the DDT-dieldrin combination at the dosages used in this experiment.

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[2] Fellow, Rockefeller Foundation.

Transmission of the Oak Wilt Fungus by *Pseudopityophthorus minutissimus* (Zimm.)[1]

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As the result of laboratory tests made at Wooster, Ohio during 1955, one instance of oak wilt fungus transmission by the bark beetle, *Pseudopityophthorus minutissimus* (Zimm.) was obtained.

In the field, this bark beetle commonly breeds in the smaller diameter branches of dying and recently dead trees of the black oak group (Griswold & Neiswander 1953). It also makes deep feeding cavities in crotches, bud axils, leaf axils and immature acorn axils of the small twigs of healthy trees of both black and

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white oak groups. Its breeding galleries are often found in the same branches occupied by galleries of *Pseudopityophthorus pruinosus* (Eichh.), another bark beetle with similar habits and with which oak wilt fungus transmission was obtained during 1954 and 1955 (Griswold & Bart 1954, Griswold 1955).

On June 8, forty freshly emerged beetles reared from non-diseased material were allowed to crawl about for one-half hour on a sporulating mat culture of *Endoconidiophora fagacearum* Bretz producing endospores only. Immediately afterward, they were transferred to a cage containing a healthy, 3-year-old potted red oak seedling having both fully and partially developed foliage. The seedling had been kept out-of-doors in a screened cage before being used in the test.

The beetles began to make feeding attacks in the seedling within 1 hour after they were transferred to the cage, and crotch, bud axil and leaf axil feeding injuries were subsequently made. When all of the beetles were dead, 12 days later, the test seedling was returned to an out-of-doors screened cage.

On July 8, six leaves on a lower branch of the seedling were beginning to discolor, curl and stiffen. By July 13, the greater number of the leaves exhibited oak wilt foliar symptoms and the seedling was cultured. On July 17, *E. fagacearum* was found growing abundantly on the cultured chips taken from both stem and root.

DISCUSSION.—Oak wilt fungus transmission was obtained by *P. minutissimus* in the laboratory through beetle contact with a sporulating mat culture of *E. fagacearum* followed by beetle feeding attack in a healthy oak seedling. It would appear probable that oak wilt fungus transmission by this bark beetle might also occur in the field should feeding attack be preceded by contact with natural, sporulating mats of the fungus growing in beetle infested branches. Such contact might occur either through mat growth into breeding galleries or through breeding gallery penetration into mats.

REFERENCES CITED

Griswold, C. L. 1955. Recent developments in the study of insect vectors of the oak wilt disease organism. Proc. Annual Meeting North Central Branch Ent. Soc. of Amer. **10**: 23–4.

Griswold, C. L., and G. J. Bart. 1954. Transmission of *Endoconidiophora fagacearum* by *Pseudopityophthorus pruinosus*. U. S. Dept. Agr. Plant Disease Reporter **38**(8): 591.

Griswold, C. L., and R. B. Neiswander. 1953. Insect vectors of oak wilt fungus. Jour. Econ. Ent. **46**(4): 708.

Piesma cinerea (Say) Feeding on Potato Foliage[1]

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Pigweed, *Amaranthus retroflexus* L., in Connecticut was heavily infested in 1955 by the ash gray leaf bug, *Piesma cinerea* (Say).[2] Numbers of this same insect were observed in several locations feeding on potato foliage. Both pigweed and potato leaves showed evident, similar discolorations where the bug had fed. The discolored areas were characterized by a loss of color in the feeding area.

Summers (1891) recorded the ash gray leaf bug, "so abundant on grape leaves and flowers as to do serious damage." Although Parshley (1923) noted that this insect was rare in the Northeast, Barber (1924), and Weiss & Lott (1924) recorded it as abundant in Massachusetts and New Jersey. Barber (1924) reported that large numbers of this bug caused serious damage to *Amaranthus caudatus*. Weiss & Lott (1924) observed that upper leaf surfaces from infested pigweed plants (*A. retroflexus*) were first mottled and later became white. Coons (1950) reported that this insect *Piesma cinerea*, transmitted savoy, a virus disease of the sugar beet. In Europe a related species, *Piesma quadratum* Fieb., spreads leaf crinkle, a beet virus disease (Wille 1928, and others). Schubert (1927) indicated that the European species did not attack potatoes.

Damage by *Piesma cinerea* was severe enough on potato plots at Windsor to justify determining the effect of sprays that were applied for flea beetle control. Insecticide applications were made on June 1 and 14 and were evaluated on June 21. In these counts the terminal leaflet of the fourth leaf down from the top of the plant was picked from 15 plants in each plot. The number of discolored feeding areas in each leaflet was recorded. The treatments in this test were in four dosage levels, each level replicated four times. A total of 52 untreated check plots were graded and averaged for comparison. Table 1 gives the results

Table 1.—Reductions of *Piesma cinerea* (Say) Damage to Potato Foliage.[a] Windsor, Connecticut. 1955.

WETTABLE POWDER	POUNDS TOXICANT PER ACRE	NO. DISCOLORED FEEDING AREAS	PER CENT REDUCTION
DDT, 50%	2.000	55	73.5
	.667	43	79.3
	.222	98	52.8
	.074	185	10.8
Dieldrin, 50%	.250	43	79.3
	.083	72	65.3
	.028	92	55.7
	.009	401	0.0
Endrin, 25%	.250	71	65.8
	.083	63	69.6
	.028	162	21.9
	.009	191	7.9
Untreated		207.5	

[a] Based on four replications of each dosage level and the average of 52 untreated plots.

of this examination. DDT, dieldrin and endrin all gave somewhat similar protection. No pretreatment count is available since this damage was not noticed until after treatment was begun.

The nature of the discoloration produced by the feeding of this bug is unknown. If this were a common occurrence on potato, it seems that it would certainly have been noticed before, due to the striking appearance of the discolored foliage.

SUMMARY.—*Piesma cinerea* (Say) was abundant in Connecticut in 1955 and fed on pigweed and potato. The area of feeding on both plants is characterized by being discolored. DDT, dieldrin and endrin applied to control potato flea beetle all reduced the numbers of feeding lesions made by this insect on potato foliage.

REFERENCES CITED

Barber, George W. 1924. Notes on *Piesma cinerea* (Say). Psyche **31**(5): 229–32.

Coons, G. H., S. E. Kotila and D. Stewart. 1950. Savoy, a virus disease of beet transmitted by *Piesma cinerea*. Amer. Soc. Sugar Beet Technol. Proc. **6**: 500–1.

Parshley, H. M. 1923. Family Piesmidae. *In* Britton, W. E., Hemiptera or sucking insects of Connecticut. State Geol. and Nat. Hist. Surv. Bull. **34**: 694–5.

Schubert, W. 1927. Biological investigations on the beet

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[2] Piesmidae, Hemiptera.

leaf-bug *Zosmenus quadratus* in the Silesian area of infestation. Z. angew. Ent. **13**(1): 128–55.

Summers, H. E. 1891. The true bugs or Heteroptera of Tennessee. Tenn. Agr. Expt. Sta. Bull. **4**(3): 75–96.

Weiss, H. B., and R. B. Lott. 1924. Notes on *Piesma cinerea* (Say) in New Jersey. Psyche **31**(5): 233–5.

Wille, J. 1928. The leaf crinkle disease of beet caused by the beet leaf bug. Arb. Biol. Abt. **16**(1): 115–67.

Kurtomathrips morrilli, a Non-Vector of the Spotted Wilt Virus, with Notes on *Liothrips urichi*[1]

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New immigrant thrips species into the Hawaiian Islands have always been scrutinized as possible vectors of the spotted wilt virus. This virus which is transmitted by *Thrips tabaci* Lind., has been sporadically but persistently destructive on crop plants in Hawaii and the possible introduction of an additional vector species would create increased difficulties. Two such cases of recent introduction, one accidental and the other deliberate, are reported here.

Kurtomathrips morrilli Moulton was first discovered in April, 1954 (Bianchi 1955), but the species determination was made later (Bianchi 1956). The infestation is, so far as observed up to the present time, limited to *Pluchea odorata* (L.) Cass., a common weed, in a localized area on the Island of Oahu. In the early days after the discovery, some local entomologists considered the possibility of utilizing this thrips species for control of the weed species, because of its gregarious feeding habit which results in killing the host plant. At the time of these considerations, however, the identity of species was not available and the extent of its injury upon cotton, bean, eggplant, and chrysanthemum (Moulton 1927, Flock 1945, Lauderdale 1946)[2] was unknown. For the interest of the Territorial Board of Agriculture and Forestry, a test herein described was conducted to determine the vector relationship of this thrips species with the spotted wilt virus.

In a feeding test, this species fed rather freely on *Emilia sonchifolia* (L.) DC., one of the major weeds in the cultivated areas and the principal weed host of the virus, but not on *Nicotiana tabacum* L. and *N. rustica* L. The length of the life cycle from egg to adult was about 30 days in November (76.5° F. average mean temperature) and about 35 days in cooler January and February (71.5° F.).

The experimental procedure for the transmission tests is the same as previously described (Sakimura 1953). Adults collected from the field-grown *P. odorata* plants were transferred to the infected *E. sonchifolia* plants. Thirty to forty days after the first transfer, the adult progeny emerging on the source plants were transferred, two per plant, to healthy *E. sonchifolia* test plants. The test plants were kept under observation for 6 weeks or longer.

During the period from November 1954 to April 1955, 252 adults recovered from 11 different source colonies were transferred to 126 test plants none of which became infected. Considering the number of the test insects and persistency among the different sources of those test insects, it is concluded that *K. morrilli* is a non-vector of the spotted wilt virus.

Clidemia hirta (L.) D. Don is a notorious weed in the tropical range and forest lands, recently established in a localized area on the Island of Oahu. To control this weed, the Territorial Board of Agriculture and Forestry, in April 1953, introduced from Fiji and released *Liothrips urichi* Karny, a well-reputed natural enemy of the weed (Fullaway 1954). As *L. urichi* has been known as a monophagus species, the possibility of this species being involved in the dissemination of the disease was considered extremely remote. Nevertheless, a cooperative feeding test, conducted together with the Territorial Board of Agriculture and Forestry, on the infected *E. sonchifolia*, *N. rustica*, and *Datura stramonium* var. *chalybea* Koch was all negative and confirmed previous observations made elsewhere. However, the susceptibility of *C. hirta* to the virus had never been determined. Prior to the introduction, an inoculation test was conducted for determining its status with the cooperation of the Territorial Board of Agriculture and Forestry. Juice inoculations of the virus on several field-grown *C. hirta* plants and replicated recovery tests from those inoculated plants were all negative. The indications were that *C. hirta* is apparently not a suscept of the virus.

REFERENCES CITED

Bianchi, F. A. 1955. Note. Proc. Hawaii Ent. Soc. **15**(3): 386.

Bianchi, F. A. 1956. Note Proc. Hawaii Ent. Soc. **16**(1): (in press).

Flock, R. A. 1945. Notes. Ariz. State Ent. Rept. for **1944**: 16, 19.

Fullaway, D. T. 1954. Note. Proc. Hawaii Ent. Soc. **15**(2): 280.

Lauderdale, J. L. E. 1946. Note. Ariz. State Ent. Rept. for **1945/46**: 17.

Moulton, D. 1927. Thysanoptera—New Species and notes. Bull. Brooklyn Ent. Soc. **22**(4): 181–201.

Sakimura, K. 1953. *Parthenothrips dracaenae*, a non-vector of the spotted wilt virus. Jour. Econ. Ent. **46**(2): 287.

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[2] The cooperation of Mr. W. T. Mendenhall, State Entomologist of Arizona, through whom these references became available to the writer is gratefully acknowledged.

Seasonal Occurrence of Resting Larvae of the Pink Bollworm in Central Texas[1]

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The most important quarantine and control measures against the pink bollworm, *Pectinophora gossypiella* (Saund.), are designed to reduce the population of resting larvae. A knowledge of the seasonal abundance of this stage of the pest in different areas where it occurs may be of value in formulating more effective control programs.

The terms "resting," "long-cycle," "overwintering," "hibernating," and "diapause" have been used to describe mature larvae that become dormant and may not pupate for several months to two or more years.

Each week during the growing season of 1955 infested green bolls were collected from various fields in central Texas, all within 120 miles of Waco. The mature larvae were removed from the bolls and placed in petri dishes, where they were permitted to spin cocoons under thin discs of absorbent cotton, according to a method previously described (Fife 1949). This method permitted observations on their development without disturbing them. From 18 to 56 larvae were placed in each dish, and they were kept in an insectary under approximately outside temperature and humidity conditions. Larvae were classified as of the resting type if they did not pupate within 30 days. Observations were also made on larval mortality after this period.

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[2] In cooperation with the Texas Agricultural Experiment Station.

Table 1.—Seasonal occurrence of resting larvae of the pink bollworm from green cotton bolls by semimonthly periods in central Texas, 1955.

PERIOD	NUMBER OF LARVAE	PER CENT OF RESTING LARVAE	PER CENT LARVAL MORTALITY
Aug. 1–15	36	3	6
16–31	156	5	3
Sept. 1–15	233	25	9
16–30	98	64	4
Oct. 1–15	153	93	4
16–30	154	94	1
Nov. 1–15	77	100	3

Resting larvae were present in all samples. As shown in table 1, very few were present during August, there was a rapid increase in September, and in October and November 93% or more entered the diapause.

The larval mortality within 30 days never exceeded 9%, indicating that conditions were favorable for further development and the larvae were not seriously injured in handling. Few of the larvae pupated after the 30-day period or after December 1, when temperatures were usually too low for further development.

Few exit holes were observed in the late-maturing green bolls during October and November, indicating that most of the resting larvae remain in the open bolls for hibernation.

To reduce the population of resting larvae in this area greatly and thereby the number of larvae that could survive to infest the next crop, it seems essential to produce and harvest an early crop and destroy the stalks by mid-September. Since this is too early in the season for practical use, cultural measures and the use of mechanical devices must be directed toward the reduction of populations after most of the larvae have entered the resting stage.

Observations were also made on the abundance of resting larvae in open bolls and locks of cotton of the 1954 crop that were collected on the soil surface or from standing plants each month from January through August 1955. In corn, alfalfa, small grain, and cotton fields, where these crops followed cotton in the rotation program, resting larvae were numerous during January, February, and March. The resting stage was also fairly numerous in alfalfa, small grain, and cotton fields during May and June, indicating survival under the various types of rotation. The last resting larva in debris from the previous crop was found on July 15.

Open bolls collected from standing stalks contained 185, 91, and 50 resting larvae per pound, respectively, on April 21, May 17, and June 10. The first open bolls collected from two fields of the 1955 crop contained 6 and 7.5 larvae per pound on July 25. Moth emergence occurred from March 17 to August 29 from infested bolls installed in hibernation cages on November 15, 1954. These results show that pink bollworm larvae in the resting stage occurred every month of the year in the central Texas area.

REFERENCE CITED

Fife, L. Courtney. 1949. Studies of the diapause in pink bollworm in Puerto Rico. U. S. Dept. Agr. Tech. Bull. 977, 26 pp.

Evidence of Lindane Translocation in Corn Plants[1]

ROBERT G. HAINES[2]

Recently there have been several reports that various plants absorb and translocate lindane. Starnes (1950) indicated that gamma-BHC was translocated in the reproductive tissues of lima beans and potatoes. Kozlova (1950) found that feeding cabbage aphid (*Brevicornye brassicae* (L.)) on cabbage plants grown in BHC-treated soil resulted in 46% mortality of the insects in 24 hours. Casida & Allen (1952), Terriere & Ingalsbe (1953), Ehrenhardt (1954), and Gladenko & Fortushynyi (1954) have also shown evidence of absorption and translocation of BHC and lindane by various plants.

In conjunction with a series of investigations[3] to determine the rates of accumulation of various insecticides in soil and the related effects on the physiological processes of vegetable crops, corn seedlings were grown in lindane-treated nutrient solution under greenhouse conditions. An attempt to study the distribution of lindane in the corn plants was made, (1) by bioassay of guttated liquid and (2) by bioassay of extracts of leaf and stem-sheath samples.

MATERIALS AND METHODS.—Corn seedlings (N. J. #7 hybrid) were selected for uniform height (6 inches) and vigor, and transferred to 1-gallon culture vessels containing nutrient solution of the Shive & Robbins (1951) formula. The nutrient supply was replenished by the drip technique and constant aeration was maintained.

Two seedlings per culture, two cultures per treatment, and four treatments plus controls were included in the experiment. The experiment was repeated at the termination of the first series. Various concentrations of lindane solution were incorporated with the nutrient stocks during the course of the experiment. The lindane solutions were prepared by dialysis using 99.99% pure lindane (Hooker Chemical Co.); the concentrations were determined both by total organic chlorine and by bioassay (Table 1.)

Table 1.—Concentration of lindane in separately prepared diffusate solutions by two different methods.

DIFFUSATE SOLUTION	CONCENTRATION OF LINDANE PRESENT	
	Total Organic Chlorine Method	Bioassay Method
Number	p.p.m.	p.p.m.
1	1.2	1.5
2	1.4	1.6
3	1.4	1.5
4	1.4	1.7
Mean	1.4	1.6

Plants of the first series were grown 64 days and treated 26 days; second series were grown 67 days and treated 22 days. Lindane concentrations of 0.25 p.p.m., 0.05 p.p.m., and 0.0185 p.p.m. were selected for the treatment range on the basis of preliminary determinations.

The small volumes of guttated liquid were collected by means of a 1-ml. graduated medicine dropper with a drawn-out tip. A length of rubber tubing was connected to the opposite end and

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[3] Soil Insecticide Accumulation and Residue Studies; an assistantship sponsored by the N. J. Agr. Expt. Station, Dept. of Entomology. Author is indebted to Drs. B. B. Pepper and A. J. Forgash for advice and aid in these studies.

the liquid collected by gentle suction. A small wad of cotton inserted in the tubing prevented dilution of the exudate with saliva. Collections of guttated liquid were made prior to sunrise. During the first series the volumes of exudate were quite small due to low humidity in the greenhouse. Consequently, humidity chambers of polyethylene sheeting were constructed to cover the cultures of the second series, thereby providing optimum conditions for guttation. Chambers were placed over the cultures during the night and removed during the day.

Samples of guttated liquid from two plants in each culture were combined and analyzed for lindane by bioassay. Bioassay organisms used were *Aëdes aegypti* larvae 66 hours old. Preliminary experimentations indicated that larvae of this age were reasonably uniform in growth, easy to handle and count, and responded to testing in a reproducible manner. Criterion for death was the inability to move away from an 8-watt fluorescent lamp placed at the bottom of each test beaker. Ten larvae in a total of 2 ml. of guttated liquid were used for each sample. The lindane content of the solution was determined from a standard mortality curve.

RESULTS.—In the first series daily volumes of guttation were below the amount necessary for bioassay and, as a result, no data were obtained. Since in the second series guttation was favored by the use of humidity chambers, ample volumes of fluid were collected. The results of the experiment are shown in table 2.

Table 2.—Average concentrations of lindane in guttated liquid collected from N. J. No. 7 hybrid corn plants grown in nutrient solution containing various concentrations of lindane (Second Series).

TREATMENTS PERIOD (DAYS)	CONCENTRATION OF LINDANE IN GUTTATED LIQUID (P.P.M.) Treatments[a]		
	0.25	0.05	0.0185
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	0	0	0
6	0.009	0	0
7	0.009	0	0
8	0.009	0	0
9	0.009	0	0
10	0.011	0	0
11	0.011	0	0
12	0.012	0	0
13	0.012	0	0
14	0.013	0	0
15	0.013	0	0
16	0.014	0	0
17	0.014	0.009	0
18	0.015	0.009	0
19	0.015	0.009	0
20	0.016	0.011	0
21	0.017	0.011	0.009
22	0.017	0.012	0.009

[a] Values are averages of two cultures. Samples of guttated fluid collected from the untreated plants each day of the treatment period were non-toxic to mosquito larvae.

At the termination of each series all plants were washed in distilled water and divided into two fractions: (1) leaves, (2) stems and sheaths. Each fraction was cut into approximately 2-cm. lengths and immediately frozen. The method for extracting lindane from the tissues was similar to that published by the Shell Chemical Company (1954). Extracts were analyzed by bioassay (Table 3).

Table 3.—Concentration of lindane in: (1) leaf blades and (2) stems and sheaths of N. J. No. 7. hybrid corn plants grown in nutrient solution containing various concentrations of lindane.

LINDANE[a] TREATMENT	CONCENTRATION OF LINDANE In Blades		In Stems and Sheaths	
	Series I	Series II	Series I	Series II
	p.p.m.	p.p.m.	p.p.m.	p.p.m.
0.25	0.21	0.17	0.61	0.50
.05	.07	.06	.11	.10
.0185	.04	.03	.05	.05
.0	.0	.0	.0	.0

[a] Length of treatment time: Series I—26 days; Series II—22 days.

DISCUSSION.—The results of this study indicate the presence of a toxic material in both guttated liquid and tissues of corn plants grown in lindane-treated nutrient solution. Although the nature of toxic material has not been demonstrated experimentally, it seems reasonable to assume that it is lindane. This assumption can be strengthened by the fact that toxicants other than lindane are very unlikely to occur. For example, degradation products of lindane are less toxic to *Aëdes* larvae than the gamma isomer (Sherman 1948), and if such were present their concentrations would be much higher than indicated since mortality calculations were based on a standard gamma isomer curve. In addition, induced production of toxic metabolites by the plants is also quite unlikely, for it seems that such an alteration of plant biochemistry would produce noticeable physiological or other differences (of which there were none) in the plant. Furthermore, if this were the case, a proportional relationship between treatments and detected toxicant would probably not exist.

SUMMARY.—A study was made to determine whether lindane is absorbed and translocated in corn plants. Corn seedlings were grown in lindane-treated nutrient solution and analyzed by bioassay. Daily volumes of guttated liquid from the corn leaves were also bioassayed. The presence of toxicity in extracts of treated samples is offered as evidence of lindane translocation.

REFERENCES CITED

Casida, J. E., and T. G. Allen, 1952. Absorption and translocation of insecticides by plants. Agr. Chem. **7**: 41–3.

Ehrenhardt, H. 1954. The effectiveness of Hexachlorocyclohexanes as systemic insecticides. Anz. Schladingskunde **27**: 1–5.

Gladenko, I. N., and V. A. Fortushynyi. 1954. Toxicity of food substances obtained from plants treated with BHC. Veterinariya **31**: 59–63.

Kozlova, E. W. 1950. Penetration of organic insecticides into plant tissues. Chem. Abs. **44**: 6563.

Shell Development Company, Agric. Res. Division, 1954. Preparation of extracts for bioassay of insecticide residues. Anal. Method: ARMS-C-6/53.

Sherman, M. 1948. Relative toxicity of the isomers of BHC to several insects. Jour. Econ. Ent. **41**: 575–83.

Shive, J. W., and W. R. Robbins, 1951. Methods of growing plants in solution and sand cultures. N. J. Agr. Expt. Sta. Bull. **636**: 1–24.

Starnes, O. 1950. Absorption and translocation of insecticides through the root systems of plants. Jour. Econ. Ent. **43**: 338–42.

Terriere, L. C. and D. W. Ingalsbe. 1953. Translocation and residual action of soil insecticides. Jour. Econ. Ent. **46**: 751–3.

A New Device for Spot Fumigation of Conveying Equipment[1]

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Effective fumigants for general or selective problems are well established. The variables inhibiting effective fumigation, in most instances, are duration of exposure, difficulty in obtaining a gas impermeable seal to retain the fumigant, and the amount of labor required in providing an effective seal. Conveying equipment, so common in grain mills, and other food processing plants, has presented perplexing problems in sealing. Some conveying equipment has been especially designed for fumigation and some is of such design or construction that sealing may be easily accomplished. Most of the older equipment in use today, however, was not built with proper access for fumigation. This principle, as well as others for sanitary maintenance were not included in earlier designs. Therefore it became imperative that modifications be established to utilize new and modern fumigants in older types of equipment. Modern fumigants efficiently minimize loss due to down equipment.

The *Fumiport*[4] was therefore devised to expedite fumigation on such equipment and, at the same time, reduce labor costs through its semi-automatic action. The gross construction of the *Fumiport* (figure 1) is essentially a base plate with a super structure of two cylinders, one within the other. The larger, or outside cylinder, has a circumference open at the bottom; its bottom edge is continuous with the base plate. This common opening communicates through the inside circumference of the base plate into the interior of the conveyor or conduit furnishing the avenue for the chemical. The smaller cylinder, inside the larger cylinder, is disposed eccentrically on the inside wall surface of the outer cylinder and is secured by a weld or by rivets which are sealed to the outside. A cotton wick is interposed between the two cylinders. The lower end extends flush with the joint at the base plate and wall of the conduit. The top portion of the wick extends over the edge of the small cylinder and reaches to the bottom of its interior. The volatile liquid is placed in the smaller cylinder, which is open at the top and closed at the bottom. A pressure type lid, sealed with vaseline, effects a gas impermeable seal at the top of the *Fumiport*. A gasket between the base plate and the conveyor wall, securely fastened, forms a tight joint at the lower end of the attachment.

A measured amount of the volatile liquid, or fumigant, is placed in the smaller cylinder or container which is open, as before mentioned, at the top. The over-all top portion of the *Fumiport*, however, is fitted with a pressure-type lid sealed with vaseline. Capillary action through the wick brings the fumigant from the inside cylinder down through the interposition of the cylinders into the large circular opening, which is synchronized with the base plate and contiguous with the opening into the wall of the conveyor or conduit.

Through the use of a *Fumiport* it is often unnecessary to do extensive sealing beyond the area to be fumigated. For example, if a given portion of conduit is to be fumigated the top and lower portion or any portion intermediate between either end may be blocked or sealed, and the *Fumiport* attached at appropriate areas. Where conduits or elevators extend into large bins or open areas, or equipment areas in poor repair, it then becomes unnecessary to do extensive sealings of these portions if spot fumigation or spraying is feasible. Such areas are not needed for introduction of the fumigant if the *Fumiport* is utilized.

The general procedure in using the *Fumiport* is to start on the lower floors of the establishment and place a pre-measured amount of the fumigant in the inside cylinder. The dosage will have been calculated for each attachment in relation to the over-all cubic feet to be fumigated. Another advantage in using the *Fumiport* is the elimination of stuffings, rags, or other inhibiting materials in the conduits to prevent excess leakage. The failure

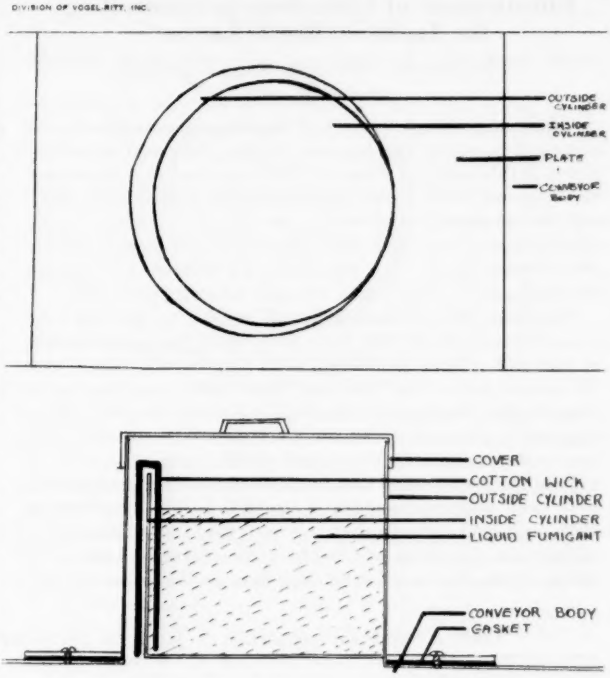

FIG. 1.—*Fumiport* device details.

to remove such rags or materials after the fumigation has accounted for many accidents.

In using the *Fumiport* the liquid fumigant does not touch the material since volatilization occurs as the liquid reaches the lower end of the wick. Thus, only the gaseous form of the chemical enters the conduit. This immeasurably reduces the possibility of product hazard through fumigation.

Aeration time and labor is reduced considerably. In aeration it is usually only necessary to open the top and the bottom of the conduit for proper ventilation. This in many cases eliminates the use of fans, opening windows, etc. on several floors when equipment cannot be properly sealed. In one particular instance, the duration of the fumigation labor required was reduced from 22–24 man hours to 7½–8 man hours. The amount of labor involved was considerably reduced since a vast amount of patch work sealing was eliminated and other areas which were accessible to spraying did not present the somewhat difficult and occasionally hazardous problem of attempting to seal through makeshift methods. The time involved in close and careful inspection to eliminate the uncertainty of sealing and relieving anxiety of accidents or other hazards is incalculable.

[1] Presented at the Twenty-seventh Annual Meeting of the Eastern Branch of the Entomological Society of America held in Baltimore Md., Nov. 21–22, 1955. Accepted for publication April 2, 1956.

[2] Industrial Sanitation Consultants, Division of Vogel-Ritt, Inc., Philadelphia, Pa.

[3] C. Schmidt & Sons, Inc., Philadelphia, Pa.

[4] Pat. No. 2,722,452.

Effectiveness of Chlordane in Controlling the Japanese Beetle Larvae[1]

J. B. POLIVKA, *Ohio Agricultural Experiment Station, Wooster*

Chlordane is accepted as one of the better insecticides for the control of larvae of the Japanese beetle. Early in the investigation of this material Fleming (1947) reported that it reduced the Japanese beetle larval population more rapidly than DDT and that 10 pounds of chlordane per acre gave results comparable to 25 pounds of DDT. However, the period of residual toxic effectiveness has not been established for different levels of application.

Chlordane was placed in field tests in Ohio for the first time as a soil insecticide in 1946. Since that time it has been included, at various levels, in 15 different tests that are distributed over the eastern half of the State and in soils that vary from sandy loam to clay. However, results of only eight of the tests will be reported in this paper. The other tests are either in their first year or were applied for the control of other grubs.

Field tests with liquid and dry applications of chlordane that have been under close study during the period from 1946 to 1955, inclusive, indicate that this insecticide is outstanding in effectiveness at rather low levels of concentration against the larvae of the Japanese beetle. The data in this paper are presented to show the comparative effectiveness of various levels of chlordane that have been in the soil for several years.

In all of these tests, chlordane was applied as a liquid or as a powder to the surface of four plots 10 by 10 feet in size at the rates shown in table 1. Liquid applications were made by mixing the amount of insecticide necessary for each plot in 3 gallons of water and applying with a sprinkling can. The dry applications were made by mixing the amount of insecticide necessary for each plot with 2 pounds of milorganite and spreading it with a small fertilizer spreader. Larval surveys were taken generally a few months after application and at yearly intervals in all tests whenever the population averaged five or more grubs per square foot in the untreated areas. Three $\frac{1}{3}$-square-foot soil samples taken to a depth of 4 or more inches, depending upon the depth of the larvae at the time of the survey, were examined for larvae in each treated and untreated plot. The results of the surveys are shown in table 1.

It is evident from the data that all levels of chlordane used in these tests were effective in controlling the larvae of the Japanese beetle. It may be noted that the poorest control was obtained the first year, or the year of application. This is due to the fact that it takes some time for the insecticide to become distributed through the larval feeding zone. A few grubs were found in plots

[1] Accepted for publication April 6, 1956.

Table 1.—Average population of Japanese beetle larvae at various times after application of chlordane.

Date of Application	Pounds per Acre	Average Population in Various Generations Following Application						
		1[a]	2	3	4	5	6	7
Sept. 6, 1946	1	6.8				0.2	6.8	2.3
	5	2.2				.0	.0	.0
	15	1.8				.0	.0	.0
	25	2.2				.0	.0	.0
	Untreated	13.0				6.6	20.0	7.5
Oct. 3, 1947	5	2.5	0.0		0.0	.0		
	12.5	.8	.0		.0	.0		
	25	.8	.0		.0	.0		
	50	.3	.0		.0	.0		
	Untreated	21.0	5.0		9.5	23.3		
Apr. 6, 1948	5		.0		.0	.0		
	20		.0		.0	.0		
	25		.0		.0	.0		
	Untreated		43.2		11.6	13.0		
Apr. 11, 1949	5		.0					
	10		.0					
	Untreated		19.5					
Apr. 11, 1949	5		.0	0.0	0.0	0.0		
	10		.0	.0	.0	.0		
	Untreated		17.5	21.0	19.8	10.0		
June 9, 1949	5	.0	.0	.0	.0			
	10	.0	.0	.0	.0			
	Untreated	13.3	12.5	15.3	5.5			
Aug. 10, 1949	5	1.3	.0	.8	.0			
	10	.8	.0	.0	.0			
	Untreated	14.8	15.0	20.3	7.3			
May 11, 1950	1				.0			0.0
	5				.0			.0
	10				.0			.0
	15				.0			.0
	20				.0			.0
	25				.0			.0
	Untreated				15.8			30.8

[a] Generation to which application was made.

of the 1-pound rate in the September 1946 test in each of the years in which the plots were sampled but even at that low level there was still some reduction in grub population. All of the other levels, including the 5-pound rate, gave 100% control consistently in all tests as shown by surveys conducted periodically for 4 to 7 years. These tests will be surveyed again if and when the larval population builds up sufficiently in the untreated areas to warrant the expenditure of time.

SUMMARY.—In eight field tests in which chlordane was used at various levels, it was found that at the 5-pound rate it gave excellent control in every test conducted and for as long as 6 years after treatment.

REFERENCE CITED

Fleming, Walter E. 1947. Chlordane for control of Japanese beetle larvae in turf. Jour. Econ. Ent. **40**(6): 932–3.

Greenbug Eggs Below the Thirty-Fifth Parallel[1]

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A considerable number of eggs of the greenbug (*Toxoptera graminum* (Rond.)), or what are believed to be eggs of this species, were found in the greenhouse at the Amarillo, Texas station during the last of March and the first part of April in 1955. According to Webster & Phillips (1912) it seemed doubtful that greenbug eggs would be found south of the 35th parallel, except in high altitudes. However, they reported that some were found in a field at Knoxville, Tenn., in December, 1908. In the north, overwintering does occur in the egg stage. Most records report that greenbug eggs are deposited in the fall. However, Webster & Phillips (1912) reported that on April 4, 1908, in Washington, D.C., males, oviparous females and eggs of *T. graminum* were observed in rearing cages.

The eggs found at the Amarillo Station had been deposited on the upper sides of the leaves of Kearney barley and a barley cross (Kearney×Ward). The plants were growing in a large flat. In most cases the shiney black eggs were found isolated on the leaf surface. However, two clusters of eggs were found; four in one cluster and six in another. Hunter (1909) reported finding eggs, deposited in a cluster, on wheat leaves. He also stated that *Toxoptera* eggs were found deposited on the sides of a breeding cage. The only aphid known to be present in the greenhouse at the time the eggs were first observed, and also during the previous year, was *T. graminum*. The eggs may have been either true eggs or aborted embryos from old agamic females. However, no eyespots or other embryonic characteristics could be detected at the time of discovery or 6 months later after a number of eggs had dried and shriveled. Part of the eggs were kept in a refrigerator for about 3 months after discovery and a number of them left in the greenhouse under dry conditions. At the end of this time, the eggs were placed in a flat of growing wheat within the controlled environment of an insectary, but none hatched. These eggs were roughly 0.5 mm. in length; somewhat smaller than the winter eggs described by Webster & Phillips (1912).

According to records available to the author, no greenbug eggs have ever been found in the field in the High Plains of Texas, but greenbugs have been found in the Hereford area during each October of the past 3 years. During the past year greenbugs were not reported in surrounding states for at least 2 months prior to October, therefore lowering the chance of local infestation from incoming migration. There appears to be a good possibility that greenbugs oversummer, in the egg stage, in some high altitude areas below the 35th parallel.

REFERENCES CITED

Hunter, S. J. 1909. The greenbug and its enemies. Univ. Kansas Bull. **IX**(2) 118.

Webster, F. M., and W. J. Phillips. 1912. The spring grain-aphis or greenbug. U. S. Dept. Agric. Bull. **110**: 44, 77, 95.

[1] Accepted for publication April 18, 1956.

The Sugarcane Beetle Damages Tobacco in Tennessee[1]

L. B. SCOTT, *Entomology Research Branch, Agr. Res. Serv., U.S.D.A.*

The adults of the sugarcane beetle, *Euetheola rugiceps* (Lec.),[2] not normally of economic importance in Tennessee, damaged isolated plantings of burley and dark fire-cured tobacco in the north-central part of the State during 1955. It is believed that this is the first record of this species attacking tobacco.

The adults damaged the stems of tobacco seedlings at or just below the soil surface. This injury was not immediately apparent when adequate soil moisture was present, but practically all the weakened plants wilted and died shortly after the onset of a mild drought. Several plantings were damaged sufficiently to require replanting.

Growers applied 20% toxaphene dust to individual tobacco plants or disked 23% aldrin emulsion into the top 3 inches of infested soil at the rate of 2 quarts per acre. Dead beetles continued to appear on the soil surface for 2 months following either treatment. Only a trace of injury occurred after the insecticides were applied.

The sugarcane beetle also damaged corn in Tennessee during 1955. The heavier infestations in tobacco were adjacent to corn infested by the same species.

[1] Accepted for publication April 4, 1956.

[2] Identified by O. L. Cartwright, Division of Insects, U. S. National Museum.

Selection of Larvae of *Anopheles quadrimaculatus* for Tolerance to DDT[1]

W. B. HAWKINS, *Division of Health and Safety, Tennessee Valley Authority, Wilson Dam, Alabama*

Attempts to develop DDT-resistant *Anopheles quadrimaculatus* Say in the laboratory have received little attention. Fay *et al.* (1949) reported that adult *A. quadrimaculatus* selectively exposed to DDT produced offsprings which were more tolerant of DDT. Three successive selections did not increase the tolerance of DDT which disappeared from the progeny of the following generation's unselected mosquitoes. In an effort to learn something of the response of *A. quadrimaculatus* to a selection pressure, larvae have been selectively exposed to DDT suspensions through five successive generations.

MATERIALS AND METHODS.—The parent strain of *A. quadrimaculatus* used in this work was established from larvae collected in 1952 at Beaver Dam Creek embayment in Kentucky Reservoir. This particular embayment, as well as nearby ones, had been routinely larvicided each summer between 1945 and 1952. These mosquitoes, the Beaver Dam (BD) colony, were maintained in the Wilson Dam insectary without treatment between the time of collection and the beginning of selection in 1955.

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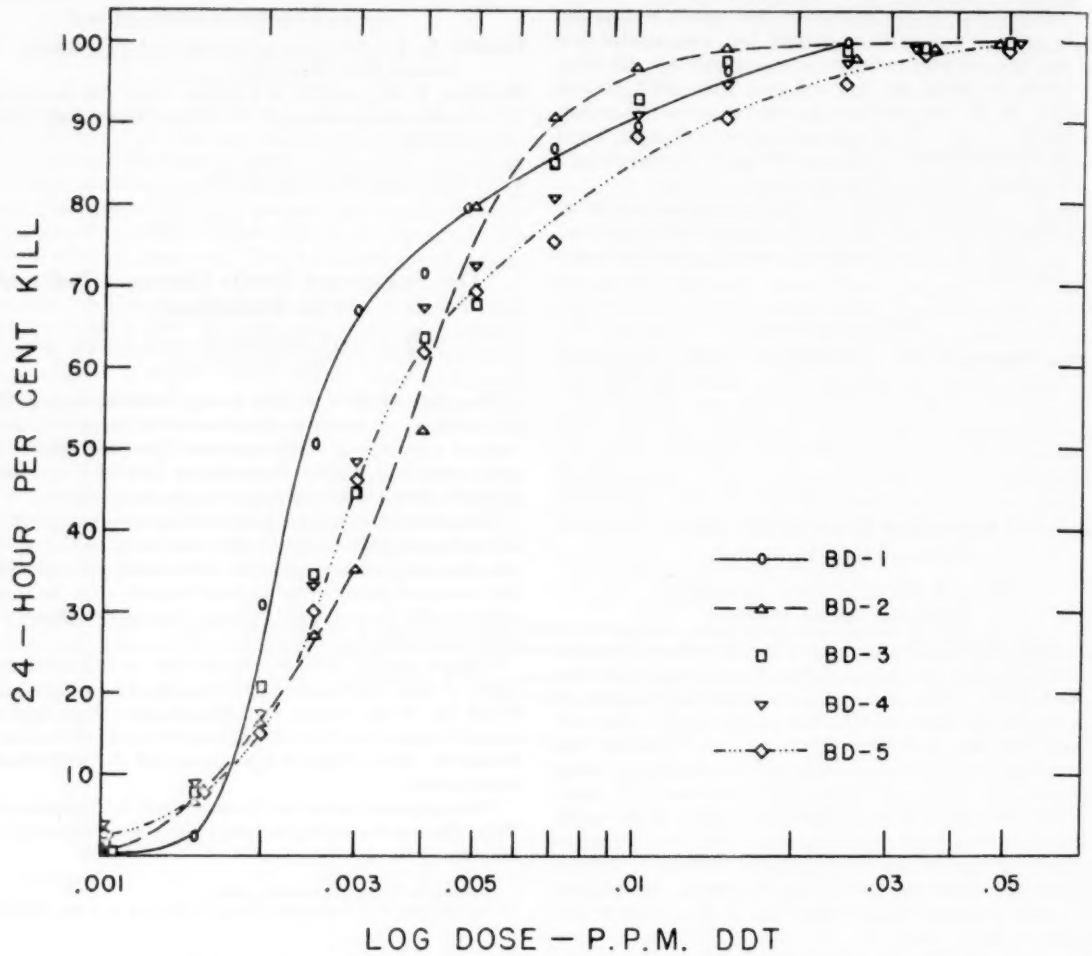

FIG. 1.—Dosage response curves for larvae of the control (BD) strain of *Anopheles quadrimaculatus*. Data for each curve gathered in parallel with that of correspondingly numbered curve of the BDR strain. The maxima in response are represented by the three curves.

The mosquitoes were reared in a manner similar to that described by Crowell (1940). The temperature of the rearing room was maintained at 75±2° F. To begin the selection, groups of 500 early fourth stage larvae were picked at random from the rearing pans and exposed for 24 hours to suspensions of DDT in polyethylene dishes. In the filial generations since only the larvae reaching the fourth instar each day were treated, the numbers of larvae available were sometimes less than 500. The suspensions were prepared by mixing 10 cc. of an alcoholic solution of DDT into 990 cc. of distilled water. The DDT concentration was adjusted from time to time in an effort to keep the kill at 90%, but the response of the larvae was sometimes erratic, and 24-hour kills ranged down to 70 and up to 100%. At the end of the exposure period, the live larvae were removed from the suspensions and put in a pan of fresh tap water where they were fed and allowed to develop. Eggs were collected throughout the life of each generation's adults. The adults of each generation were maintained in separate cages. This sub-colony of mosquitoes produced by selective exposure of the larvae to DDT will be referred to as the Beaver Dam-R (BDR) colony.

To compare the response to DDT of the BD and the BDR strains, dosage mortality data were collected on larvae from each generation of the BDR colony, and in parallel tests on larvae from the BD colony. The larvae were exposed for 24 hours to graded doses of DDT as suspensions made up in "pyrex" glass dishes by adding 1 cc. of an alcoholic solution of DDT to 99 cc of distilled water. The temperature of the suspensions was held at 75±2° F. Twenty-five larvae were used in each replicate. At least 200 larvae of each colony were tested at each of 13 dosages during a generation.

Because of skewness, a log-probit transformation did not yield a straight line for much of the dosage response data. The curves for the later generations of the BDR colony could, however, be satisfactorily transformed. Though the data are believed to be sufficiently quantitative to merit a probit analysis, a simple plot of kill versus log dose will illustrate the significant features of the results.

DISCUSSION OF RESULTS.—The dosage mortality data for the larvae of the Beaver Dam colony are plotted in figure 1. It will be noted that there is some variation among the series of tests. The LD_{50}'s range from 0.0025 to 0.0038, while the LD_{90}'s range from 0.007 to 0.015 p.p.m. Except for the larvae of the F_1 generation of the BDR colony, those of the later generations show some increased tolerance of DDT (fig. 2). A comparison of the data for the larvae of the BD colony and those of the F_1 generation of the BDR colony shows that five successive selections with DDT have increased the LD_{50} from 0.0025 to 0.0038 p.p.m. DDT to about 0.0076 p.p.m. DDT. By the same comparison the LD_{90} has increased from 0.007 to 0.015 p.p.m. DDT to about 0.025 p.p.m. DDT.

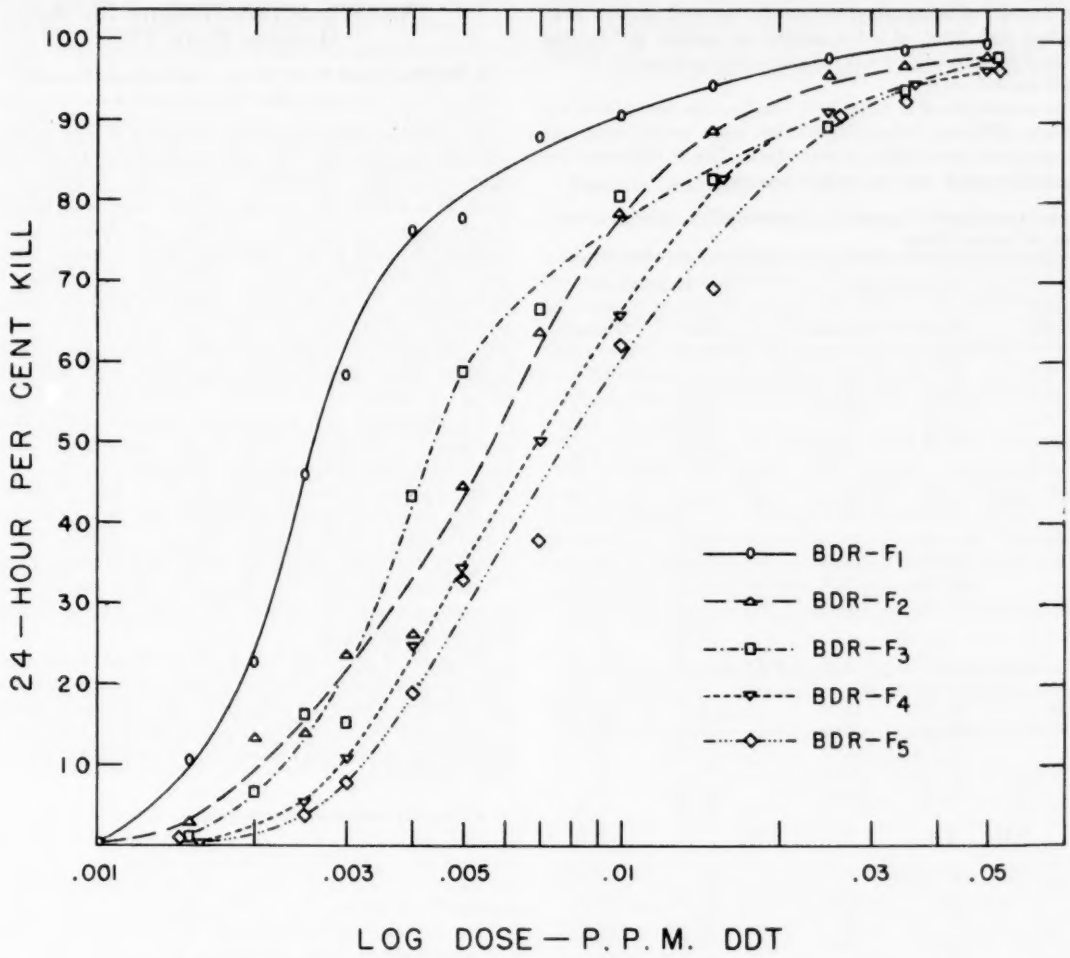

FIG. 2.—Dosage response curves for each generation's larvae of the selected colony (BDR).

These increases in LD_{50}'s and LD_{90}'s of the BDR larvae have resulted, not from a shifting of the curves throughout their length, but roughly from a rotation about the 100% kill point such that the slopes of the curves have been increased. According to Hoskins & Gordon (1956) such increases in slope may only denote a greater vigor among the survivors of a selection. They also suggest that a population, which responds to selection as have these *A. quadrimaculatus*, probably lacks factors necessary for the development of true resistance. Thus, though conclusions must be drawn with restraint because of the small number of selective exposures, the data suggest that *A. quadrimaculatus* may be incapable of developing resistance to DDT.

REFERENCES CITED

Crowell, R. L. 1940. Insectary rearing of *Anopheles quadrimaculatus*. Amer. Jour. Hyg. 32(1): 12–20.

Fay, R. W., W. B. Baker, and Mary Grainger. 1949. Laboratory studies on the resistance of *Anopheles quadrimaculatus* to DDT and other insecticides. Jour. Natl. Malaria Soc. 8(2): 137–46.

Hoskins, W. M., and H. T. Gordon. 1956. Arthropod resistance to chemicals, in Annual Review of Entomology 1: 89–122. E. A. Steinhaus and R. F. Smith, Editors, Annual Reviews, Inc., Stanford, California.

The Copper Content of Resistant and Susceptible House Flies[1]

SAMUEL J. RINGEL, *Entomology Research Branch, Agr. Res. Serv., U.S.D.A.*

The enzyme tyrosinase, a copper-protein complex, is associated with the darkening and hardening of arthropod cuticle (Richards 1951). If, as reported by Wiesmann (1947), resistant house flies (*Musca domestica* L.) are more deeply pigmented than susceptible ones, it seemed probable that they might contain more tyrosinase and hence more copper.

To determine whether such a difference does exist, susceptible and resistant house flies were analyzed for copper. The susceptible flies were from the normal laboratory colony. The resistant insects were the offspring of the Orlando colony developed by Lindquist & Wilson (1948), the ancestors of which had been exposed to DDT for more than 200 generations. With the exception of the exposure of the resistant flies to DDT, the regular Chemical Specialities Manufacturers Association rearing procedure (Anonymous 1951) was used.

The insects, separated according to age and sex, were killed by exposure to cold temperatures and analyzed, in groups of 10, for copper by a slight modification of the short diethyl dithiocarbamate method (Association of Official Agricultural Chemists

[1] Accepted for publication April 4, 1956.

1955). Instead of being ashed in a muffle furnace, the flies were wet ashed with 3 ml. of a 1:1 mixture of sulfuric and fuming nitric acid until the digest was colorless. After ashing, the AOAC method was followed.

From results given in table 1, it can be seen that there is a significant difference in copper content on a weight basis between male and female flies in both strains but no difference between the resistant and susceptible insects.

Table 1.—Copper content[a] of susceptible and resistant strains of house flies.

Age (days)	Susceptible			Resistant	
	Male	Female		Male	Female
1	13.5	15.7		10.1	15.1
	18.6	10.5		9.8	7.3
	8.6	14.2		9.5	5.3
	10.8	9.1		8.6	6.7
		7.5		9.4	5.5
					8.1
S.D.[b]	3.7	3.1	S.D.	0.5	3.3
2	8.3	12.3		10.7	5.1
	6.9	14.5		10.9	3.4
	6.9	8.2		8.6	5.1
	8.4	8.1		6.8	7.5
				7.3	6.9
				12.9	7.1
S.D.	0.7	2.7	S.D.	2.2	1.4
3	23.5	11.3		9.4	6.1
	20.5	9.9		11.8	6.4
	21.6	12.1		10.3	6.4
	22.9	12.4		10.2	7.9
	20.3	13.0		12.6	7.1
				10.9	8.3
S.D.	0.4	1.1	S.D.	1.1	0.8
4	20.6	7.4		31.6	24.3
	17.7	10.8		32.9	16.9
	17.0	6.7		20.2	13.2
	19.3	8.2		24.1	15.2
	17.1			18.9	13.5
	13.3			25.1	21.5
S.D.	2.3	1.6	S.D.	5.3	4.1
5	12.3	9.7		18.3	9.9
	13.4	8.7		14.5	8.3
	11.8	6.3		18.3	7.8
	17.1	8.4		10.1	8.6
	12.5	8.2			9.3
	14.0	7.4			
S.D.	1.8	1.1	S.D.	3.4	0.7

[a] Micrograms per gram.
[b] Standard deviation.

References Cited

Anonymous, 1951. Peet-Grady method. Soap and Sanitary Chem., Blue Book and Cat. Ed., pp. 237–43.

Association of Official Agricultural Chemists. 1955. Official methods of analysis, 8th ed.

Lindquist, Arthur W., and H. G. Wilson. 1948. Development of a strain of house flies resistant to DDT. Science **107**: 276.

Richards, A. Glenn. 1951. The integument of arthropods. University of Minnesota Press.

Wiesmann, R. 1947. Untersuchungen über das physiologische Verhalten von *Musca domestica* L. verschiedener Provenienzen. Schweiz. Ent. Gesell. Mitt. **20**: 484–504.

An Oviposition Medium for the Mexican Fruit Fly[1]

M. McPhail and F. E. Guiza, *Entomology Research Branch, Agr. Res. Serv., U.S.D.A.*

To meet the demand for large numbers of eggs in connection with the mass rearing of the Mexican fruit fly, *Anastrepha ludens* (Loew), a highly satisfactory oviposition medium was developed. It is a dome-like, orange-colored, reinforced, hollow hemisphere made of cheesecloth, paraffin, petrolatum, and coloring matter, prepared in the following manner: Spread cheesecloth over a flexible form (rubber ball, handle of a rubber syringe, citrus fruit), dip to required depth in a mixture containing 3 parts of paraffin (m.p. 51.6°–52.8° C.) and 1 part of petrolatum, with chrome color added to produce orange shade; cool, apply a second layer of cheesecloth, dip again, cool, brush down to bring out outline of cheesecloth, remove from form, and trim.

To obtain eggs with these domes, the procedure followed depends on the object of the collection. In an ordinary oviposition experiment, place the domes as such among the flies or paraffin them to pieces of glass and expose to the flies. If the object is rearing, then the inside of the domes must be kept moist to prevent the eggs from drying out. This is accomplished by placing a wad of wet cotton under the dome or by atomizing the inside of the dome with water. The fly punctures the dome and deposits its eggs on the inside surface where they can be conveniently brushed or washed out.

The color of the dome is important. Shape is also important. The cheesecloth serves two purposes. It reinforces the dome and side in the mechanics of oviposition. Petrolatum is added to the paraffin to bring about the required softness. Repeated use of these oviposition media for periods of 6 months has not impaired their usefulness.

[1] Accepted for publication April 24, 1956.

Spraying Cloth Patches with Fabric Protectants[1]

C. R. Moreland,[2] *Pesticide Testing Laboratory, Department of Agriculture, Ottawa, Canada*

One of the purposes of the Pesticide Testing Laboratory is to evaluate various commercial fabric-protectant insecticides. The candidate products must be applied according to the label directions and may not be diluted or otherwise modified. The product should be applied at the same rate as the standard insecticide with which it is compared.

These principles make it necessary to evaluate dosage in terms of total deposit rather than as residue after evaporation of the solvent. Uniformity of application over the surface of the cloth is an essential. Pipetting the insecticide is impractical for dosages in the range of 25 mg. per square inch, and dipping is not satisfactory unless acetone or some other diluent is permitted. In any case, since most labels direct the user to spray the garment, a spraying technique is indicated.

The American Society for Testing Materials (Anon. 1953) and Chemical Specialities Manufacturers Association (Anon. 1952) textile resistance tests are for pretreated fabrics and yarns and give no method for applying insecticides. It is known that some laboratories treat swatches of cloth and later cut out suitable test patches, but it was decided here to cut patches first and then

[1] Accepted for publication May 1, 1956.
[2] The author wishes to express his appreciation to Mr. W. S. McLeod for his assistance with the spraying and also for his aid in the preparation of the manuscript. Mr. A. E. R. Downe gave valuable suggestions.

spray them. Circles of 2 square inches of Botany-style No. 315 broadcloth containing 100% wool[3] served as test patches.

The early work on a technique of spraying patches was done on a conveyor table on which the patches moved through the spray pattern from a pneumatic atomizing nozzle. Patches within a replicate received uniform dosages, but it was not possible to achieve uniformity between consecutive replicates. Since table speed, air pressure, and liquid pressure were constant, it was assumed that the difficulty was due to the nozzle, and a new technique was sought.

It was decided to use the spray chamber, constructed after Bottimer (1945) and described in the Cockroach Spray Method in Soap and Chemical Specialites Blue Book (Anon. 1955), with a DeVilbiss Special No. 5004 atomizer. The intake tube of the atomizer was bent at 90° for easy aspiration from shell vials (Hazard 1945). The 18-inch square chamber was fitted with a sliding Plexiglas front panel. A pressure of 6 ± 0.5 p.s.i. was used throughout the studies.

A weighing bottle was weighed on an analytical balance. A cloth patch was added and the value recorded. The patch was then centered on the hardware cloth 28 inches below the nozzle, and a known dosage was aspirated from a shell vial. The patch was inverted and sprayed with a similar dosage. The patch was quickly placed in the weighing bottle and reweighed to determine the deposit. Slight adjustments of the dosage were often necessary before required deposits were obtained.

In one particular investigation, it was necessary to apply total dosages of 10, 20, 30, 40, and 50 mg. per patch with an allowable range of ± 2 mg. Eight replicates of each dosage, a total of 40 patches, were required. This number was obtained by spraying 67 patches and selecting the 40 most satisfactory ones. The volume pipetted into the shell vials ranged from 0.25 to 0.55 ml. in this series, and equal amounts were applied to both sides of each patch.

In general, patches were sprayed in groups of five, and total deposits within the group varied within a range of less than 10 mg. from the least to the greatest. Occasionally a value would fall far out of the range. A 1-ml. pipette graduated in tenths was used, but a pipette with finer graduations would be an advantage.

At the commencement of spraying the atomizer was primed (Hazard 1945). After each patch was sprayed, the nozzle was cleared by placing a finger over its tip. The hardware-cloth floor was wiped with absorbent paper or acetone between tests. The exhaust was reduced to a minimum (approximately 10 feet per minute) in order to prevent disturbance of the mist.

REFERENCES CITED

Anonymous, 1952. CSMA textile resistance test. Soap and Sanitary Chem. Blue Book pp. 259–262, 276.

Anonymous, 1953. Tentative methods of test for resistance of textile fabrics and yarns to insect pests. D 582-49 T. ASTM Standards on Textile Materials pp. 111–116.

Anonymous. 1955. Cockroach spray method. Soap and Chem. Spec. Blue Book pp. 255, 256.

Bottimer, L. J. 1945. Roach rearing and testing. Soap and Chem. **21**(12): 151, 153, 155, 157, 159.

Hazard, F. O. 1945. Modifications of the liquid roach method. Soap and Sanitary Chem. **21**(12): 159, 167.

[3] Standard mothproofing test fabric of the American Association of Textile Chemists and Colorists, and of the Chemical Specialties Manufacturers Association.

Potato Leafhopper Control on Snap Beans[1]

CHARLES H. BRETT and ROSS W. BRUBAKER[2]

Insecticide tests against bean insects have been made in the mountain area near Hendersonville, N. C., for a number of years. One species to appear each year has been the potato leafhopper, *Empoasca fabae* (Harr.). During the season of 1955 this insect developed an unusually heavy population coincident with a late cool spring and heavy rainfall. The visual effects of its presence on bean plants and differences between the insecticide treatments were striking.

The test procedure consisted of dusting plots of snap beans with a double-outlet hand crank duster at the rate of about 25 pounds per acre. Dusts were applied July 15 and 19. Leafhopper nymphs were counted on five trifoliate leaves from each of the two center rows in each plot. Counts were made July 21, 28, and August 4. Since each treatment was replicated four times this resulted in a total of 12 counts per treatment.

Table 1 shows Perthane, methoxychlor, and Strobane to be much more effective than malathion or rotenone. Rotenone was the least active material. Mixtures of malathion and Perthane or malathion and methoxychlor indicated no synergistic effect, but rather a dilution of insecticidal activity by the presence of the less active malathion.

Table 1.—Potato leafhopper control on snap beans using dusts applied at the rate of about 25 pounds per acre. Fletcher, N. C., 1955.

DUST	CONCENTRATION (PER CENT)	PER CENT CONTROL
Perthane	5	88
Methoxychlor	5	80
Strobane	20	78
Malathion plus Perthane	3 3	74
Malathion plus Methoxychlor	3 3	62
Malathion	4	30
Rotenone (Cube)	1	16
Rotenone (*Tephrosia vogelii*)	1	11

[1] Accepted for publication May 1, 1956.

[2] North Carolina Agricultural Experiment Station and U.S.D.A., Agr. Res. Serv., Entomology Research Branch, respectively.

EDITORIAL

Professional Standards for Entomologists

If trends in other scientific disciplines are an indication, a matter that will require ever increasing attention from the membership of the Entomological Society of America is the development of adequate professional standards for entomologists. From observations of the development and present status of such organizations as the American Chemical Society, the American Society of Civil Engineers, the Society of American Bacteriologists, and the Society of American Foresters, it is apparent that the establishment of professional standards is a pronounced evolutionary trend in the American scientific society.

It may be correctly said that the Entomological Society of America already possesses professional standards, since the constitution of the Society restricts active membership to persons engaged in work in entomology or allied fields and to such other persons as have suitable training or interest in entomology. It is true that this requirement imposes a certain limitation on membership, but it is not sufficiently limiting to check the rather widespread attitude among engineers, chemists, medical personnel and others, that entomologists are highly specialized technical experts whose services are needed as assistants but who do not normally qualify competitively for project administrators or similar high levels of professional employment. Accordingly, for the purposes of this discussion, professional standards are conceived to be requirements for specified minimal levels of didactic training and performance capability, collectively so designed as to insure an ultimate improvement in the general level of professional practice.

If we grant, for the sake of discussion, that the mass of entomologists recognize the need for more exacting professional requirements, what avenues of approach are there to the supply of this need?

It is believed the first and most practical approach is to draft a definition of an entomologist for the constitution of the Society which requires more than a simple expression of interest in insects. In this advanced age, it would not seem out of line to require all new "Active Members" of the Society to have a bachelor's degree in entomology from a recognized institution of higher learning. Variations of this provision could be written to include students and, if desired, the unusual individual with informal training and experience equivalent to or greater than a bachelor's degree. Furthermore, to retain the active affiliations of pest control operators, specialists from other disciplines with an abiding interest in entomology, and the frequent dedicated individual who contributes solely from an inborn scientific zeal, an "Affiliate Member" status requiring a certain number of demonstrable years of interest related to entomology could be established. However, regardless of these secondary variations, our recognition of the fact that formal training is required for the efficient practice of entomology would constitute a significant advance in the eyes of our professional associates from other scientific professions.

Perhaps the most cogent and least individually-biased criticism of the above-described approach is the fact that the Entomological Society of America was established, and is operated, as a "scientific society" and not as a professional organization largely dedicated to the betterment of the welfare of the membership. It is not believed these objectives are mutually exclusive. However, if it develops that there is an overwhelming desire to maintain our organization in this former status, a second and equally effective approach to the development of professional standards could be through the establishment of a suitable form of "certification" for those segments of the Society where the need is greatest. For example, it is believed that such a need particularly exists in the subspecialty of medical and veterinary entomology, since individuals employed in this field customarily work beside physicians, veterinarians, and public health sanitarians—all members of groups with exacting requirements for practice. Space does not permit the elaboration of specific details for the development of certification in this and other specialities. In general, however, it is conceived that it might be accomplished by a board organized within the framework of the Entomological Society of America and having as its principal function the conferring of professional rating upon successful applicants by means of a certificate indicating the possession of special training and experience.

What tangible results may be expected from the development of professional standards? The following are some of the advantages which it is believed would develop: (a) the establishment of true professional standards would make it possible for all interested outside agencies to determine who, in the eyes of their own colleagues, are qualified and competent in the field of entomology; (b) for the first time, adequate professional criteria would be available to prospective employers of entomologists; (c) the quality of academic training would eventually be profoundly influenced; and (d) it is believed that greater professional opportunities for entomologists would inevitably result.

It is freely recognized that there are disadvantages in such a program. For example, it is conceivable that the establishment of formal academic requirements might create a gulf between some segments of the society and could possibly even cause a loss of membership. To the argument that the erection of professional standards might exclude highly esteemed entomologists who have come to their present standing without formal academic training, it can be replied that any such plan must contain a "grandfather clause" permitting the automatic exemption of all present membership.

It is recognized very fully that the establishment of adequate professional standards for American entomologists will not be an easy problem. Such establishment spells change to present ways of thinking and doing, and change inevitably creates unpleasant situations for some. However, change has a way of forcing itself upon us, whether desired or not, and this brief discussion is simply a plea for recognition of a problem which already faces some segments of our Society. As a practical approach, it is believed that a permanent committee on "Status and Standards of Entomologists" for the Entomological Society of America could guide the society in these matters and serve as a medium through which needed revisions or amendments could be made to the constitution and bylaws.

KENNETH L. KNIGHT, *Cdr. (MSC) USN*
Bureau of Medicine and Surgery,
Department of the Navy

OBITUARIES

George Adam Dean
1873-1956

George A. Dean, one of America's leading entomologists and for 31 years Head of the Department of Entomology at Kansas State College, died April 23, 1956, at the age of 83, after a long illness.

Professor Dean, as we all referred to him, was born April 19, 1873, at Topeka, Kansas. He received his B.S. and M.S. degrees from Kansas State College in 1895 and 1905, respectively. He was awarded an honorary Doctor of Science degree by Southwestern College at Winfield, Kansas, in 1943. He also attended Kansas State Normal College, Emporia, in 1897–1898, and secured a state teacher's certificate in 1898, after which he was Principal of Highland Park grade school at Topeka. In 1902, he was appointed Assistant in Entomology at Kansas State; and in 1912, he became Head of the newly organized department, Entomologist of the Kansas Agricultural Experiment Station, and State Entomologist in the Kansas Entomological Commission, which positions he held until his retirement in 1943. From 1923–1925, he was on leave of absence to head the Division of Cereal and Forage insect investigations in the Bureau of Entomology in Washington. He was a member of the Mediterranean fruit fly Commission from 1928–1930 which supervised the eradication of this pest from Florida.

Under Professor Dean's administrative leadership, the heat method for control of insects infesting flour mills was developed and the county wide campaign plan of using bran mash for the control of grasshoppers, cutworms, and armyworms was first demonstrated in 1913 to 1918. By virtue of his position, he served as State Entomologist for the north half of Kansas on the Kansas Entomological Commission and continued quite active in regulatory work until 1945. He earned the reputation of being a good teacher and generally made special preparation for each recitation or lecture.

Professor Dean was president of the American Association of Economic Entomologists in 1921, the Entomological Society of America in 1925, the Kansas Entomological Society in 1930, and the Kansas Academy of Science in 1937. He was a Fellow of the American Association for the Advancement of Science.

Other organizations to which he belonged included Sigma Xi, Alpha Zeta, Phi Kappa Phi, Gamma Sigma Delta, and the Farm House fraternity.

He was recognized for service to the College at a Zoology and Entomology banquet October 2, 1937, and at an alumni dinner May 31, 1947. A more detailed account of his life and work (with a listing of 60 of his publications) was given in the "Summary of Twenty-five years of work in the Departments of Entomology and Zoology at Kansas State College" (Kans. State College Bull. 21(9): 55 p. Oct. 1937).

He was survived by one son, Paul, an architect, of Englewood, Colo.; and three daughters, Helen Elisabeth Dean, Worthington, Ohio, a librarian at Ohio State University; Mrs. Harold L. Nonamaker, Manhattan; and Mrs. Ralph L. Miller, Tulsa, Okla. He was preceded in death by his wife, the former Minerva Blachly, who died last December, and by a son, George T. Dean, who died in 1952.

Professor George A. Dean
(Taken in 1950)

Those of us on the staff who were associated with Professor Dean since the 1920's appreciated the freedom and cooperation given us in our teaching and research. He endeavored always to build up and maintain a strong department. He was especially anxious to reach more students and to serve the public with our science. Under his direction, the first full-time Extension Entomologist in Kansas, was appointed, thus beginning a major public education service in the State in Entomology.

Professor Dean was held in high esteem nationally particularly for his judgment, hence his advice was sought by many. His outstanding characteristics were his honesty and fine Christian character, his industriousness, punctuality, his fine personal appearance, and his exceptional memory. He left with us a pattern of hard work, of exemplary personal habits and morality, and complete devotion to our profession.

ROGER C. SMITH

Royce Burton Knapp
1924-1955

Royce Burton Knapp, 31, entomologist with the Cereal and Forage Insects Section, Entomology Research Branch, U. S. Department of Agriculture, at Beltsville, Md., died at Washington, D. C., on December 9, 1955.

Born at Binford, N. Dak., on June 1, 1924, Royce was the son of Olaf and Susan (Shaw) Knapp. He attended grammar and high school at Binford, N. Dak., and the North Dakota Agricultural College, graduating with a B.S. degree in 1946. From 1946 to 1948 he attended graduate school at North Dakota and also assisted in teaching entomology. While at the College he conducted research and was author or joint author of several papers dealing with livestock pests, wireworms, and the wheat stem sawfly. In 1948 he accepted an appointment in the Cereal and Forage Insects Section of the Entomology Research Branch and conducted research on the wheat stem sawfly at Minot, N. Dak., the European corn borer at Ankeny, Iowa, and on legume insects at Beltsville, Md.

On June 14, 1946, he was married to Betty Jean Getman, who survives. While they had no children, Mr. Knapp was affectionately called "Uncle Louis" by many children in his home neighborhood. He had a great fondness for the outdoors and was an enthusiastic hunter and fisherman. He was also an expert square dancer.

Mr. Knapp was a member of the Entomological Society of America, of the Entomological Society of Washington, of Sigma Alpha Epsilon social fraternity, and Phi Kappa Phi honorary society. He was also a member of the Moose and Elks lodges.

Mr. Knapp was at Beltsville only a few months but had made a wide circle of friends. His associates held him in high regard and will long remember his cheerful and friendly disposition.

B. A. APP

Trunk Sprays for Peach Tree Borer Control[1]

W. D. WYLIE, *University of Arkansas, Fayetteville*

A single application trunk spray showed promise as a method of control for peach tree borer, *Sanninoidea exitiosa* (Say). A heavy dosage of dieldrin applied in June appeared to prevent successful establishment of borer larvae in the tree throughout the remainder of the season.

The peach tree borer is a serious pest in Arkansas. If uncontrolled it is capable of destroying the usefulness of an orchard. Many trees are killed outright and the remainder are weakened sufficiently to make them susceptible to attack by other insects and by diseases.

Fumigation of trees in the fall with paradichlorobenzene has been fairly successful in Arkansas. Disadvantages of this method of control include the amount of labor involved in making the application and the fact that considerable injury occurs prior to treatment. Trunk spraying to prevent entry of newly hatched borers has become widely accepted in many peach growing areas. This type of control has not been particularly successful in Arkansas. The long period of time over which adults are active makes it necessary to have protection for approximately 5 months. Where the residual life of the spray deposit is only 3 weeks, an excessive number of applications are needed to obtain control.

The trunks of a number of peach trees were sprayed June 21, 1955 at Clarksville. The sprays were applied with a small power sprayer using 200 p.s.i. The spray contained 6 lb. to 100 gal. of 50% dieldrin wettable powder. Approximately 3 pints of spray material was used to spray the trunk from ground level up to the main scaffold limbs. Sprayed trees were alternated with unsprayed trees. Indications of recent entries were present on the trees when the spray was applied. A second application of dieldrin was made to part of the sprayed trees on August 5. A number of trees were also sprayed for the first time on that date. These latter trees were well infested when the sprays were applied. An additional block of infested trees was sprayed for the first time September 9, in the same manner as those sprayed earlier.

Observations were made periodically throughout the season on the sprayed trees and the adjoining unsprayed trees. No new entries were evident in the trees sprayed June 21. On September 8, several sprayed trees were artificially infested with large numbers of eggs of the peach tree borer. On September 27 there were indications that a few new entries had occurred on these trees. On that date no other evidence was seen of borer activity in the trees sprayed June 21. The number of borers in the trees sprayed September 9, did not differ appreciably from the number on untreated trees, which averaged 12 borers per tree. The trees were examined again March 6, 1956. At that time the borer populations averaged six borers per tree in the untreated trees. No evidence of any borer activity could be found in the trees sprayed with dieldrin on June 21, 1955. Presumably the borers entering the tree from the artificial infestation on September 9 had failed to survive.

The results to date from the use of a single application of dieldrin early in the season are very encouraging. Further work is needed for verification of these results as well as to establish more firmly the timing and dosage required.

[1]Accepted for publication April 25, 1956

Publication Policies and Manuscript Rules and Suggestions for the Journal of Economic Entomology

The Governing Board of the Entomological Society of America has defined the fields of interest for the *Annals of the Entomological Society of America* and the JOURNAL OF ECONOMIC ENTOMOLOGY as follows:

"*The Annals.* The areas of interest for this publication should include the fields of classical entomology sometimes termed 'academic,' such as taxonomy, biology, morphology, and normal physiology. Contributions accepted need not have as their objective immediate economic application or utility.

"*The Journal.* The areas of interest for this publication should include such fields as chemical or biological control, surveys, regulation, toxicology, and abnormal physiology. In general, contributions accepted should have as their objective, sometimes indirectly perhaps, practical application or utility.

"In the case of papers which might go to either publication the editors should be governed by the historical character of each publication, and the objectives, expressed or in context, of each paper."

The Governing Board of the Entomological Society of America unanimously approved the following resolution:

"BE IT RESOLVED that the Governing Board approves the policy that no manuscript which has been published elsewhere, either wholly or in essential part, will be accepted for publication in the ANNALS OF THE ENTOMOLOGICAL SOCIETY OF AMERICA or the JOURNAL OF ECONOMIC ENTOMOLOGY. Authors submitting papers to these journals are earnestly requested to cooperate in the administration of this policy."

It should, therefore, be understood that articles submitted for publication in the *Journal* have not appeared previously elsewhere, and that they will not be offered for simultaneous publication in other journals without the consent of the Editor of the JOURNAL OF ECONOMIC ENTOMOLOGY.

Papers acceptable for publication shall in general consist of progress or final reports on original biological or biochemical research and its application in the field of applied entomology and closely related fields, including quarantine activities. In general, review articles containing little or no previously unpublished information are not acceptable for publication in the *Journal*. Invitation papers presented at the general sessions of the Society or compilations of real interest to a large part of the membership may be accepted if presented in acceptable form. Other papers dealing with economic entomology, such as papers dealing with important insect problems, or reports on large-scale control activities may be accepted at the discretion of the Editor with the approval of the Editorial Board. In the consideration of papers, an important factor shall be the value of such papers in the field of economic entomology. The primary criteria for approval of an article for publication will be the scientific merit of its content and the clarity and conciseness of its manner of presentation. If the Editor's decision is challenged, he shall refer the matter to the Editorial Board.

Preferably, each paper should be accompanied by the written approval of one or more persons familiar with the subject. Most papers are read and criticized by one or more reviewers. The author is subsequently given an opportunity to revise his paper in accordance with the reviewers' comments. If revisions are made all pages of the original manuscript, as well as any retyped ones, must be returned to the Editor in order to receive consideration. Only heavily corrected pages need to be retyped, instructions for which are discussed under "Preparation of Manuscripts." This procedure is not intended to imply any reflection on the merits of individual papers but is followed solely for the purpose of improving the quality of articles appearing in the JOURNAL OF ECONOMIC ENTOMOLOGY and to reduce publication costs. Papers are unacceptable if conclusions are obviously unsound or based on faulty experimental methods or inadequate data. Acceptable papers must be in accord with the rules and suggestions printed in the *Journal*. When the Editor or the Editorial Board finds that a paper is unacceptable it will be returned to the author promptly with an explanation for not accepting it.

Papers, including Scientific Notes, which bear the names of any non-member authors who derive their livelihood in the field of entomology may not be accepted for free publication in the *Journal*. This requirement shall not apply to invitation papers presented at the general sessions of the Society. If they are of general interest and otherwise acceptable, they may be published free. Other papers by non-members, if sufficiently meritorious, may be published free upon approval by the Editor and the Editorial Board. Co-workers in other fields of science who are members in good standing of their respective national organizations are not required to join the Society in order to publish papers occasionally under joint authorship with members. Student membership has been provided for individuals entering entomology as a profession and those eligible should be encouraged to take advantage of such membership. Authorship should be restricted to those who *actually planned and conducted* the work reported. Meaningless authorship should never be given.

All papers by members of the Society accepted for free publication shall be published in the approximate order of their receipt in final form for publication by the Editor. Prompt publication of acceptable matter, by non-members as well as members, may be obtained by payment of publication costs at the rates specified under "Publication Costs." The date of receipt of articles or scientific notes in acceptable form shall be published with each paper. Scientific notes are accepted for publication in the approximate order of their receipt independent of that of other papers. Scientific notes shall be indexed and their titles shall be included in the table of contents.

No paper that will occupy more than 20 printed pages in the *Journal* will be accepted for publication without approval of the Governing Board.

A paper accepted for publication which would occupy less than one and one-half pages in the JOURNAL OF ECONOMIC ENTOMOLOGY in 9 on 10 point type, including

illustrations and tables, shall usually be published as a Scientific Note. Preliminary studies or fragmentary data, if they furnish good leads, should be presented for use as a Scientific Note. Authors' preference, if any, on borderline cases should be expressed when the paper is submitted for publication but it shall be the prerogative of the Editor to determine where it shall appear in the *Journal*. Descriptions of equipment and methods used in entomological research should be included in the reports on the research in which they were employed and should not be submitted separately as Scientific Notes unless unique or of special interest to many readers of the *Journal*.

Preparation of Manuscripts. The following suggestions are presented in order to minimize revisions and editorial corrections. Uniformity of style and format necessitates close attention to detail by authors even though many points may seem arbitrary. The cooperation of authors in the preparation of manuscripts will permit more prompt publication of a greater number of papers with the funds available.

Papers containing data on unknown chemicals, designated only by code, symbol, or generic name must have the composition fully disclosed or they will not be accepted for publication in the JOURNAL OF ECONOMIC ENTOMOLOGY. Authors should describe clearly and specifically the materials with which they are working, giving their nature and their composition. In reporting experiments with formulations such as wettable powders and emulsifiable concentrates authors should give the concentration of the active ingredient and in indicating dosages make it clear whether they are in terms of the formulation or of the active ingredient. In reports of certain experiments it may also be desirable to include other ingredients in the formulation.

Use of the common names of insecticides and the interim designations of insecticides which have been approved by the Committee on Insecticide Terminology of the Entomological Society of America is mandatory in the JOURNAL OF ECONOMIC ENTOMOLOGY. Use of the common names of insects which have been approved by the Committee on Common Names of Insects of the Entomological Society of America is also mandatory in the JOURNAL OF ECONOMIC ENTOMOLOGY. Authors are urged to consult the latest published lists before preparing their manuscripts for the *Journal*.

Scientific names of insects and other animals should be checked for accuracy and completeness. The author of every species mentioned should be given, in parentheses if necessary, at least once in the text of every manuscript. Scientific names of plants should be given in conformity with accepted botanical usage.

The title of a paper should be short and concise. The manuscript should be clearly typewritten on opaque paper—8½×11-inch sheets, on one side of the paper only. The lines should not exceed 6 inches in length. All typed material should be double spaced. *Do not use single spacing* for footnotes, legends, quoted matter, references cited, etc. These materials are almost always more difficult to set and to proofread than straight text and should not be made more difficult by single spacing. Corrections in the typed copy should always be made at the same level as the line in which they are to appear. When an addition is made, cut the sheet and paste the insert in its proper place. Only brief inserts of a word or two should be attempted in the margin; the printer works with the typescript mounted in a frame that exposes only a few lines at a time.

The ribbon copy of the manuscript is required. It should be mailed flat; not folded or rolled. Pages should be numbered consecutively and should not be stapled or pinned. Author's complete address to which proof is to be sent should appear at upper right-hand corner of first page. Do not underline any words except names of genera and species and to indicate the use of italics for special emphasis. Number footnotes consecutively with Arabic numerals, except in tables, where lower case letters should be used. Footnotes are referred to by means of superscript numerals or letters. Text footnotes should be typed double spaced, in consecutive order on a separate sheet of paper and put at the end of the manuscript. Sub-headings intended to be set by themselves in the middle of a page should be avoided. Appropriate side headings should be used; not more than two per typewritten page meet most needs. They should be informative, not mere labels or tags.

Tables are expensive. They should always be reduced by the authors to the simplest form. Tables should not be used if text or illustrations give the same information. Tables should be typed double spaced, on separate sheets of paper, one table to a page, numbered consecutively, and placed in a group at the end of the manuscript. Give each table a clear, concise heading stating its contents. When headings for a number of tables are similar, an attempt should be made to combine the tabular data thereby avoiding repetition of the same heading. Use a double horizontal line immediately below the title of the table and a single horizontal line below the column headings and at the bottom of the table. Do not use horizontal lines in the interior of the table. Use no vertical lines. Tables should be arranged so that, regardless of their length, the number of columns is reduced to the minimum. Papers submitted with more tables than seem necessary, or with tables poorly arranged, will be returned to the author for modification.

Illustrations should be clear and unquestionably suitable for reproduction. They are accepted only when they contribute materially to the information presented in the paper. Graphs and other line drawings should be so constructed that the greater dimension is vertical rather than horizontal. Lines on the drawings, as well as letters and figures, should be heavy enough to stand reduction without losing their clearness. Waste space should be kept to a minimum. Drawings should be on pure white paper or light blue cross-hatched paper, for satisfactory reproduction. Tracing paper or cloth is satisfactory where there may be an advantage in its use. Good photographs, when available, should accompany obituaries.

Illustrations are run as text figures designated by Arabic numerals. Units of a composite illustration are designated by letters. Arrange full-page figures to conform to *Journal* proportions (6⅛×9 inches, including legend), making due allowance for reductions. Magnification stated in the figures or in the explanation of the figures should refer to the magnification after reduction. A scale may be drawn on the figure which will automatically

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Publication Costs. Prompt publication of acceptable matter may be secured by payment toward the cost of printing and engraving ($16.00 per page plus the cost of engravings with additional charge for excessive tabular matter). Cost of engravings in excess of $15.00, accompanying each eight printed pages or major fraction thereof, will be charged to the author.

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These prices are effective for reprints of all papers accepted after January 1, 1956 for publication in the JOURNAL OF ECONOMIC ENTOMOLOGY and are in accordances with action of the Governing Board taken at the Annual Meeting in Cincinnati, Ohio, November 28–December 1, 1955.

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Grasshopper fossil of the Jurassic Age, 140 million years ago. Photo courtesy American Museum of Natural History, New York.

Here yesterday . . .

here tomorrow?

In a very old Book, it is written "The land is as the Garden of Eden before them, and behind them a desolate wilderness, yea, and nothing shall escape them." (Joel 2:3) The reference is to grasshopper damage.

In the United States, grasshoppers inflicted heavy crop losses as early as 1797. In 1877, grasshoppers alone were causing 2 million dollars' damage to crops each year. In addition, many other kinds of crop pests were making serious inroads on all types of commercial and food crops. Growers were constantly faced with the spectre of near or total crop destruction.

Today . . . thanks to modern chemical discoveries . . . things are different! Pesticides such as aldrin, dieldrin, endrin, D-D®, and Nemagon are powerful weapons in the "battle of the bugs." They kill fast. In some uses, they kill for months and years after application. These pesticides were born of years of research and vast expenditures of money. But research never stands still!

Even now, as these pesticides take their place as leaders throughout the agricultural industry, Shell Chemical has new pest-killing chemicals in the experimental stage. In time, they too will be ready for effective commercial application. And perhaps Mr. Grasshopper may indeed be "here today, gone tomorrow."

SHELL CHEMICAL CORPORATION

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